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THE STANDARDIZATION OF ANTIPNEUMOCOCCUS AND ANTIMENINGOCOCCUS SERUMS *

AUGUSTUS B. WADSWORTH, M.D.,
MARY B. KIRKBRIDE AND RUTH GILBERT
ALBANY, N. Y.

During the latter part of 1916 the laboratory received reports indicating that some of the commercial antipneumococcus and antimeningococcus serums which had been purchased in the state lacked potency when used in the treatment of cases of pneumonia and meningitis.

Specific regulations for the standardization of diphtheria antitoxin were prescribed by an act of Congress in 1903, and following this, in 1907, the immunity unit for measuring the strength of tetanus antitoxin was officially defined and the method of standardization published. Since that time, and especially during the last four or five years, while the production of different immune serums such as are produced by immunization with the pneumococcus, streptococcus, meningococcus and the dysentery bacillus has been greatly extended, no federal regulations establishing standards of potency have been formulated for these serums. The federal license to sell these serums has been issued solely on the basis of an inspection of the manufacturer's plant and tests of the purity of the serums. No tests of the potency of the serums were made until the last few months of 1918, and no standards of potency have yet been formulated or established.

Ever since the summer of 1915 this laboratory has produced antipneumococcus serum, and was therefore fully equipped to test samples of this serum which were purchased; but it was only in July, 1916, that the laboratory undertook the production of antimeningococcus serum, so that careful studies were necessary in order to acquire the technic necessary to develop methods which could become accept-

* From the Division of Laboratories and Research, New York State Department of Health.

able standards. The results of these tests of the serums produced by different laboratories, and our experience in formulating standard methods for the state are considered of sufficient interest to record, despite the fact that none of the methods by which these serums can be tested is wholly satisfactory or comparable to those used in testing the antitoxins of diphtheria and tetanus.

ANTIPNEUMOCOCCUS SERUM

Early Protection Tests of Antipneumococcus Serum.—While protection tests were early recognized as affording the most satisfactory means of testing the potency of pneumococcus immune serum, confusion was for some time caused by the irregular and conflicting results obtained by different workers due, as we now know, in a large measure to their more or less indiscriminate use of organisms belonging to the homologous or heterologous groups. To Neufeld chiefly is due the recognition of this source of error, and the present methods on which are based the standardization of antipneumococcus serum. He first classified as irregular strains, all those which did not correspond with the culture which had been used extensively by him, and which he designated as typical. Later he recognized at least three other types and, owing to the frequent occurrence of these atypical strains, he laid great emphasis on the importance of identifying each culture by serologic tests.

Cole and his co-workers at the Rockefeller Hospital have since, by their classification of the pneumococcus, developed a working procedure of great practical value in the standardization of serum as well as in the rapid diagnosis of types.

Neufeld, Römer, Wadsworth, Washburn and others in testing the potency of immune serum used rabbits extensively. These were generally given intravenous injections of immune serum followed, after an interval, by injections of homologous culture. Or, in other instances, the injection of live organisms preceded that of the serum (Wadsworth). It was found, however, that the longest period between the injections of a virulent culture and the effective administration of the serum was approximately four hours.

Six years ago the results of studies of the action of immune serum in the treatment of pneumococcus infection of the rabbit were published (Wadsworth) which demonstrated that the true therapeutic value of such serums should be determined not by their protective action, but by their curative action when given after pneumococcus infection had developed. Distinctions were then drawn between

the parasitism[1] of the agent and its toxin production. The action of immune serum was found to be very largely limited to neutralization of the toxins or poisons. The destruction of the pneumococci in the lesions whether partial or complete was secondary.

In order to demonstrate experimentally beyond question the therapeutic value of the serum, this method of testing was essential; but as a basis for the standard method of testing the degrees of potency in different antipneumococcus serums with the necessary precision, it is far from practical now that the therapeutic value of a high protective titer is so well established.

As early as 1909 Neufeld developed what he considered a satisfactory method for the standardization of serums, using mice weighing between 18 and 20 gm. instead of rabbits. He injected subcutaneously or intravenously 0.2 c.c. of the serum to be tested, followed after an interval of two or three hours by varying amounts of a twenty-four hour broth culture given intraperitoneally. He originally held that the mice should survive at least from seven to ten days, but later reduced the time to three or four days, and in his later tables recorded results only up to the forty-eighth hour. He recorded that satisfactory protection could be obtained against 0.1 c.c., and even against 0.2 c.c. of culture, but emphasized the importance of having the culture of high virulence. Neufeld also suggested that the culture and serum might be given simultaneously, and that a standard serum of known potency might be used as a control.

Results of repeated agglutination tests made at this laboratory had early shown that the relationship between the protective and the agglutinative titers were inconstant at times. While the agglutinative titer may be high during the early months of immunization, a gradual decline may and frequently does occur, while the protective titer remains stationary or even advances.

When the work was first begun in our laboratory a careful study was made of the different methods of determining the protective titer of immune serum in the hope of gaining greater precision in demonstrating slight differences in the therapeutic potency. Numerous

1. Parasitic activities of the pneumococcus are the disturbing elements not only in determining the action of the serum therapeutically, but in determining the potency of the serum by protection tests. It is difficult to conceive of any fundamental difference existing in the pneumococcus poisons except quantitatively in the different cultures. The parasitic activities, however, vary greatly in degree, constituting the chief variable factor and one which it is extremely difficult to control. The differentiation of the organisms of the different types is based entirely on differences in the bacterial cell, and has not yet been demonstrated to comprise also qualitative differences in the pneumococcus poisons or toxins; in fact, the differences in the therapeutic, and possibly the protective action of homologous and heterologous serums might conceivably be attributed to differences in parasitic activity of the organisms.

changes in the method were tried. A standard dose of the culture containing a relatively small number of fatal doses and varying amounts of serum in corresponding greater dilutions, and then smaller quantities of serum as a standard dose, were all tested in a series of preliminary investigations which, however, were sufficiently complete to warrant the conclusion that further and prolonged study and practical experience with any new methods would be necessary before they could be adopted. Although these investigations have not yet been completed, the only important change in the methods as they have been used at the Rockefeller Hospital that gave greater precision in the test was the use of 0.1 c.c. of serum instead of 0.2 c.c.; 0.1 c.c. of the serum having frequently protected against as large a dose of the culture as 0.2 c.c., showing that when the larger dose was used an excess of serum was present. This accounts for the fact that 0.1 c.c. of serum instead of 0.2 c.c. was used in many of the tests where the doses of standard culture ranged from 0.1 c.c. to 0.5 and even 0.6 c.c.

Antipneumococcus serums which were produced by different laboratories were obtained, and the protective potency of all of them was tested. The results of all of these tests are recorded in Table 1. The laboratories are designated by letters, and the letters which have been used correspond to those which were used by Amoss to designate the same laboratories in his tests of the antimeningococcus serums.

As compared with the serums of laboratories G and H, those of Laboratories C, E and F, with one exception, were all so far below standard as to fail to show protective value with the small dose of culture which was used in the test. The one exception was the second sample of serum produced by Laboratory E. The first serum of this laboratory had, however, previously failed in the test. The laboratory was notified at that time and immediately took precautions to send for cultures and protocols of our methods; they had no difficulty in producing a satisfactory serum by the time the second sample of serum was examined. The serums of Laboratories G and H of this table, and Laboratory B not listed in the table, maintained uniformly in these and other tests a high degree of potency. These laboratories were all research laboratories not primarily engaged in manufacture for sale. The serums of Laboratories C, E and F were all commercial serums purchased in the open market. Although licensed to sell, not one of these commercial laboratories produced a satisfactory Type I serum until their attention was directed to their shortcomings.

Thus, as a direct result of these potency tests of commercial antipneumococcus and antimeningococcus serums offered for sale in the State of New York, and also following reports that certain commercial serums lacked therapeutic value, the Commissioner of Health,

feeling considerable responsibility in the matter, called the attention of the Public Health Council of the state to the situation. The council acted promptly, passing the following regulation of the Sanitary Code:

CHAPTER IX. Regulation 1. Sale of antipneumococcus and antimeningococcus serum regulated. No serum for the treatment of pneumonia or of meningitis shall be sold or offered for sale in the State of New York unless each package is accompanied by a label or circular on which is stated the potency of the serum as tested by the methods established by the rules and regulations of the state commissioner of health; and no such serum shall be sold or offered for sale the potency of which does not equal or exceed the minimum fixed in such rules or regulations.

This regulation will become effective February 15, 1918.

TABLE 1.—THE RESULTS OF TESTS OF THE POTENCY OF ANTIPNEUMOCOCCUS SERUM PREPARED IN DIFFERENT LABORATORIES

		TITER OF PROTECTION TESTS IN MICE				
Control Mice: Culture without serum 0.000001 c.c. killed mice in less than forty-eight hours. Cultures: 18 hour Witte peptone broth cultures made from recent semisolid stock cultures.						
Laboratory	Date Tested	Type I Neutral Culture Homologous Culture of Serum Produced by Laboratory H		Type I R. D. Culture Heterogenous Culture of Serum Produced by Laboratory H		Remarks
		Quantities	Protection	Quantities	Protection	
H	3/29/16	0.1 serum 0.5 culture	+	Not tested	..	Serum clear
G	3/29/16	0.1 serum 0.5 culture	+	Not tested	..	Serum clear
E	1/10/17	0.2 serum 0.1 culture	0	Not tested	..	Serum clear
H	1/10/17	0.1 serum 0.3 culture*	+	Not tested	..	Serum clear
H	8/1/17	0.1 serum 0.6 culture	+	0.2 serum 0.2 culture	+	Serum clear
H	9/13/17	0.1 serum 0.4 culture	+	0.2 serum 0.1 culture	+	Serum clear
C	9/13/17	0.1 serum 0.2 culture	0	0.2 serum 0.1 culture	0	Serum slightly turbid; sterile upon tests
E	9/25/17 and 10/3/17	0.1 serum 0.3 culture	+	0.2 serum 0.1 culture	+	Serum very turbid; hemolytic; sterile upon tests
F	9/25/17	0.1 serum 0.1 culture	0	0.2 serum 0.1 culture	0	Serum slightly turbid; hemolytic; sterile upon tests
H	9/25/17	0.1 serum 0.5 culture	+	0.2 serum 0.2 culture	+	Serum clear

* Made as a control, therefore, not tested above 0.3 culture; for upper limits of potency see other tests.

This, we believe, is the first time a state has prescribed and made effective regulations for testing biologic products which are offered for sale.

RULES AND REGULATIONS FOR THE TESTING OF THE POTENCY OF ANTIPNEUMOCOCCUS SERUM [2]

I. Type of Serum.—Antipneumococcus serum for the treatment of pneumonia which is offered for sale in the State of New York shall be prepared by the immunization of animals against pneumococcus of Type I.

II. Minimum Standard of Potency.—The potency of the antipneumococcus serum intended for therapeutic use shall equal that of the standard serum prepared by the Laboratory of the State Department of Health and which is supplied on request. The standard serum is of such potency that under the conditions prescribed, 0.2 c.c. will protect a mouse 16 to 22 grams in weight for at least 4 days against at least 0.1 c.c. of the standard culture of pneumococcus Type I, 0.000001 c.c. of which will kill the control mouse in less than 48 hours.

III. Standard Cultures.—The standard culture of pneumococcus Type I employed for testing purposes may be obtained from the Laboratory of the State Department of Health. However, before the protection tests are performed, the virulence of the culture shall be determined by experiment to be such that 0.000001 c.c. of an 18-hour broth culture will kill a mouse of 18 to 22 grams weight within 48 hours.

IV. Method of Testing the Potency of the Serum.—The dilution of the culture and of the serum shall be made in broth and prepared immediately before the test is performed and in such manner that no less than 0.5 c.c. are measured. Thus 2 c.c. of the serum are diluted with 3 c.c. of the broth, so that 0.5 c.c. of the mixture will contain 0.2 c.c. of the serum. Similarly 1 c.c. of the culture is added to 4 c.c. of the broth, so that 0.5 c.c. of the mixture will contain 0.1 c.c. of the culture.

In making the injection 0.5 c.c. of the diluted culture and 0.5 c.c. of the diluted serum are thoroughly mixed in the barrel of the syringe and in two minutes injected into the peritoneum of a mouse weighing from 16 to 22 grams. Moreover, control tests with standard serum and control tests of the virulence of the culture without the serum are made at the same time. The protective titer of the serum against the culture is the potency and should be regarded as equal or in excess of the standard serum.

V. Physical Properties.—The serum shall be free from all the cellular or solid elements of the blood. In order to facilitate inspection, the serum should be put up in containers of colorless transparent glass.

VI. Sterility of the Serum.—Sterility tests of the final product shall comply with the requirements of the U. S. Public Health Service.

VII. Expiration Date.—Expiration date for the sale of the serum shall comply with the requirements of the U. S. Public Health Service.

ANTIMENINGOCOCCUS SERUM

A similar investigation was also made of the antimeningococcus serums which were produced by these different laboratories. Before this was completed Amoss tested independently serums produced by all of these laboratories, using in these tests practically similar methods, but Amoss used only selected strains publishing the results he obtained from five standard cultures of the meningococcus, whereas a much larger number of strains were used in the production of each of the serums, and it might well have happened that some of the strains were not used in the immunization of the horses at the time the serum was taken from them. It was thought that the results of the tests of all the strains used in the immunization of our horses would afford a safer basis of comparison.

2. These rules and regulations are subject to revision from time to time.

The testing of the potency of antimeningococcus serums has always been a difficult problem owing to the unsatisfactory results of protection tests, and the difference of opinion as to the therapeutic value of other antibodies which may be present in the serum.

Kraus and Doerr used bacterial extracts that were toxic for guinea-pigs and attempted to test the protective value of serums against them, but their results were not constant owing to both the variability in the toxicity of the extracts and the resistance of the different pigs.

Many workers have attempted to use live cultures for protection tests. Under the direction of Park a large series of tests were made, but a minimum lethal dose for guinea-pigs could not be determined, as there was so much variation in the reaction of the different pigs in a series. Flexner and Amoss obtained better results by using small pigs weighing from 90 to 100 gm.; but even with these, variations in resistance were met with. Hitchens reports fairly constant results if very young cultures are suspended in dilute guinea-pig serum and white mice used in making the protection tests. The practical value of his method, however, has not yet been established.

The other antibodies in the serum have been studied by a large group of workers. Among them, Neufeld investigated the bacteriotropic substances, Hohn the bacteriolytic, and Kolle and Wassermann the bacteriotropic, antitoxic, agglutinating, and complement fixing properties. Neufeld, Krumbein and Schatiloff, Baecher, and Hachla concluded that the complement fixing, agglutinating, and *opsonic* substances in the serums did not run parallel. Kolle and his helpers did not find the complement fixing and the bacteriocidal properties present in equal amounts. Later workers, however, have not confirmed these results. Amoss and Wollstein determined that the *opsonic,* complement fixing and agglutinating properties of a serum ran parallel. They tested the serums from their horses by all three of these methods until this was determined. They then relied wholly on the agglutination test to determine the value of the serums.

Amoss reported that the statistics which he had been able to gather from the use of the serums distributed from the Rockefeller Institute indicated that the serums which possessed a high agglutinating titer gave excellent therapeutic results, and that frequently when complaints had been received regarding the failure of the serums to give satisfactory results in the treatment of cases the serum did not possess high agglutinating titer. Such reports as we have received are also to this effect.

Attempts to formulate protective tests with the meningococcus having not as yet come into general use, it has been necessary to use either the agglutinating or complement fixing properties of the serum,

although these do not necessarily bear quite so clear and definite a relation to the therapeutic value of the serum. A test by complement fixation does not differentiate satisfactorily the degree of activity which the serum possesses with different strains of the meningococcus. There are many indeterminate technical difficulties in the preparation of bacterial antigens. Certainly the standardization of antigens which will give complement fixation that is indicative of therapeutic potency has not yet been accomplished. The significance of the agglutination

TABLE 2.—The Results of Tests of the Potency of—

Agglutination Titer at 55 C.

Serum	Date Tested	Stock Cultures																													
		1	2	3	4	5	6	7	8	9	10	30	31	32	33	35	36	37	38	39	40	41	42	43	44	44A	45	60			
A	Sept. 1916	0	0	0	10	0	0	10	10	0	10	10	0	...	10	0	0	10	50	0	0	0	10	10	0	10	...	0			
B	Sept. 1916	50	50	50	50	200	100	50	500	50	200	100	100	...	100	50	50	200	500	50	50	50	500	50	50	200	...	100			
C	Sept. 1917	50	50	50	50	...	50	200	500	800	100	200	...	10	100	10	50	200	500	800	10	50	800	100	10	....	50	800			
D	Sept. 1916	10	100	50	100	50	10	10	100	50	200	50	50	...	50	50	10	100	200	10	50	10	100	50	10	200	...	100			
	Sept. 1917	0	0	10	0	...	0	500	100	10	500	100	...	100	0	0	200	200	500	100	0	0	100	0	0	....	800	50			
E	Sept. 1916	100	50	50	100	50	50	10	100	50	50	100	50	50	100	100	100	200	200	100	50	100	500	100	100	500	...	100			
	Sept. 1917	100	100	800	100	800	100	800	10	10	800	800	500	200	200	200	200	...	800	800	50	10	800	50	100	....	500	500			
F	Sept. 1916	10	10	10	10	10	10	10	200	10	100	50	10	100	10	10	10	50	100	50	50	10	50	10	10	100	10	...			
	Sept. 1917	100	200	50	100	100	50	50	10	0	500	200	50	50	200	10	50	...	100	800	50	0	800	50	50	....	50	100			
G	Sept. 1916	200	100	200	200	200	500	100	500	200	200	500	500	200	200	200	100	200	500	200	500	100	500	50	100	200	...	200			
	Sept. 1917	100	800	800	800	800	800	800	800	800	800	50	800	800	500	200	200	...	500	200	200	200	800	100	500	....	800	500			
H	Sept. 1917	200	500	100	200	200	500	200	500	500	500	500	500	200	500	200	500	500	200	200	200	200	100	100	500	....	200	800			
I	Sept. 1916	10	10	10	10	200	50	10	200	10	100	100	50	100	50	10	50	100	200	50	50	50	50	10	50	20	...	10			

Agglutination Titer at 38 C.

A	June 1917	0	0	0	0	0	0	0	0	0	0	...	0	0	0	....	0	0	0	0	0	0	...	....	0	0					
D	June 1917	10	50	50	10	10	50	10	...	50	50	50	...	50	10	10	....	10	100	10	50	100	50	50	...	....	50	50			
E	June 1917	10	10	0	50	50	50	10	...	50	50	50	...	50	200	10	....	50	200	50	10	50	100	50	...	....	50	50			
F	June 1917	10	50	200	50	10	50	10	...	100	500	10	...	50	100	50	....	100	500	100	50	200	200	100	...	....	200	50			
H	June 1917	10	100	200	50	200	50	50	...	500	200	200	...	100	500	100	....	200	500	100	100	100	10	50	...	....	100	100			

may also be questioned. This question assumes greater importance since in our experience the agglutinating titer of antipneumococcus serum does not always run parallel with its protective value.

Clinically, experience in this country and abroad has demonstrated the necessity of using serums produced by strains of the homologous types of the meningococcus, and it is now suggested that it may be necessary to base treatment on the bacterial diagnosis of type, and then use the homologous serum. If polyvalent serum, serum produced by immunization with a large number of strains, is considered essential, the potency of the serum must necessarily be tested by the agglutina-

tion method. Although agglutination and complement fixation are both used, few, if any, laboratories have sufficient confidence to rely on complement fixation alone without some control by agglutination against the different strains of the meningococcus which are used in the immunization of horses.

Although many important technical changes have been introduced in our methods of determining the agglutinating titer of antimeningococcus serums since the tests of the commercial serums were made,

—Antimeningococcus Serums Prepared in Different Laboratories

Agglutination Titer at 55 C.

Stock Cultures																					Appearance
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	
0	0	.....	0	0	10	0	0	0	0	0	0	0	10	10	0	0	0	0	0	0				
0	10	.....	200	100	200	200	100	100	50	200	10	50	500	100	200	50	100	50	50	10				
100	10	.....	800	10	800	500	100	100	10	800	100	100	10	200	100	10	50	10	50	10	10	...	100	Amber colored; slightly cloudy (Sept. 1917)
10	10	.....	100	50	50	10	50	10	50	10	10	10	200	10	50	10	10	10	10	10	...	...	...	Amber colored; very
10	0	10+	50	10	50	10+	100	0	200	50	200	0	200	200	200	0	0	200	0	100	0	200		cloudy (Sept. 1917)
50	100	.....	...	500	100	200	200	100	200	200	500	100	200	500	200	100	200	100	100	200	...	...	...	Amber colored; slightly
100	50	.....	800	200	800	800	100	200	100	800	800	800	800	800	800	800	500	800	800	10	200	...	10	cloudy (Sept. 1917)
0	10	.....	50	10	50	10	...	50	10	200	...	10	100	200	50	10	50	50	10	0	...	...	...	Amber colored; slightly
50	100	.....	50	10	800	50	100	50	0	800	10	200	200	800	200	800	800	800	800	100	10	...	10	cloudy (Sept. 1917)
10	100	.....	...	200	200	200	200	100	200	500	200	100	200	200	200	200	...	200	100	200	...	...	...	Straw colored; clear
500	500	800	200	200	800	800	200	200	100	200	200	200	200	500	500	200	800	...	200	500	200	...	100	(Aug. 1917)
200	200	500	500	100	800	200	100	200	800	200	100	100	...	100	100	100	100	...	100	100	100			
0	10	.....	50	10	100	50	...	10	10	200	10	50	200	200	50	100	200	50	50	10				

Agglutination Titer at 38 C.

0	0	0	0	0	0	10	...	...	0	0	0	0	0	0	0	0	0	...	0	0	0			
10	10	0	50	10	50	50	...	...	50	10	10	50	10	10	50	10	100	...	10	10	10			
0	50	0	10	50	50	200	...	...	100	50	50	10	10	50	100	50	200	...	50	10	50			
0	50	50	50	200	100	500	...	...	200	200	50	100	100	100	200	200	500	...	100	50	100			
10	50	50	50	500	100	50	...	...	200	100	100	200	200	50	200	100	200	...	500	50	50			

the methods which were used in these tests correspond closely with those which were used by Amoss of the Rockefeller Institute when he tested the commercial serums of these same laboratories against five standard cultures of the meningococcus.

In Table 2 is recorded the agglutinating titer of all of these serums with practically all of the cultures which were used in this laboratory. The laboratories are designated by letters which correspond to the letters which were used by Amoss to designate the same laboratories.

It is evident from these results that the agglutination of the different cultures and the agglutinating action of the different serums on

the same culture as well as on the different cultures, vary greatly, and that in the absence of any standard set of cultures representative of the different types or groups of the meningococcus, and in the absence of any standard serum or standard methods with which to make the tests of potency, it was necessary to test the serums with all of the strains which were used in immunizing the horses. Although the results of our tests confirm the general conclusions of Amoss, one or two of the serums might be credited with a higher potency than they actually possessed if results from any five of the cultures were selected as a basis for comparison. This is especially noteworthy on account of errors in the classification of strains as normal, irregular, or parameningococci. In fact, the classification of a few of the strains which were used in our tests according to this grouping have since been found to be faulty. Some of the variation in the action of the serums was attributed to the fact that when such a large number of strains were used in immunizing the horses, the amount of culture taken from each of them for the inoculation was varied from time to time, one strain giving greater reactions in the smaller doses than another strain. It was necessary to increase or diminish the dose as the agglutinating titer rose or fell. This procedure becomes increasingly complicated when cultures which fail to react with the polyvalent serum are continually added to the set of cultures which is used for immunizing the horses, but in the light of the results previously tabulated showing such marked variation in the agglutination with different cultures of the homologous group, it is apparently necessary and for the present indispensable to continue this practice. We now have an experiment under way testing the effect of immunization with but four strains selected as representative of the different groups of the meningococcus, and it is possible that with these four cultures a polyvalent serum will be obtained which will possess sufficient potency when tested with many if not all of the strains of the different groups.

On the basis of these tests of the antimeningococcus serums, the commercial and noncommercial laboratories are not quite so strikingly differentiated as compared with the results of the tests of antipneumococcus serums. Laboratories B, G and H are research laboratories, and have usually been found to produce serum of fairly uniform and satisfactory potency, but in the table the serum of Laboratory B in this instance was an exception. Laboratories A, C, D, E, F and I are commercial laboratories. The serums which they produced were far from uniform, excepting those of Laboratory A which were in both instances no more potent than normal horse serum. The first serum of Laboratory E, September, 1916, as compared with the second, September, 1917, shows striking differences in potency. The same is true of

the two serums of Laboratory F. When the first tests of this table were completed, it was considered necessary to direct the attention of Laboratories A, C, D, E, F and I to the fact that their serums lacked potency by this method of testing, and this may account for the improvement in some of the serums.

It is thus very evident that a uniform method of testing the potency of antimeningococcus serum should be adopted, but the problem of formulating a standard method which could be used by the different laboratories and give reliable results was not so simple with antimeningococcus serum as it was with antipneumococcus serum. Many technical difficulties were encountered. A method of preparing standard suspensions for the agglutination reaction had to be devised and tested. A standard serum to be used for purposes of comparison in all of the tests had to be selected, and finally, standard cultures had to be chosen, and all of this without delay.

The staff of the British Army working under Gordon have differentiated four types — Type I corresponding to the Rockefeller parameningococcus, Type II corresponding to the normal or regular group, and Types III and IV are included in the Rockefeller irregular group. Gordon and those who have been able to confirm his results consider this classification complete with all of the strains of the meningococcus which they have been able to isolate and classify by both agglutination and absorption tests.

Other British research workers under the direction of F. W. Andrewes serving the civil population, agree more closely with the American grouping. They reached the conclusion that there are two main groups, (1) including the Gordon Types I and III, and (2) corresponding to the Gordon Types II and IV. These two main groups can be further divided into subgroups.[3]

For the present, in our work the American classification has been adopted because certainly two groups can be definitely recognized if all of the strains which fail to correspond in their reactions with these two groups are classified as irregular or atypical strains.

In choosing the four standard cultures representative of the different types of the meningococcus, it was decided to take one culture from the normal, one from the para, and two from the irregular strains; but the first task was to test all of the strains against monovalent serums by the agglutination and absorption tests in order to be certain of their identity and classification. The monovalent rabbit

3. There is a discrepancy in the use of the terms "para" and "normal" in the classification of meningococci. The workers at the Rockefeller Institute apply the term "para" to organisms belonging to the Gordon Type I, and the term "normal" to those in the Gordon Type II; while workers in England and France refer to organisms belonging to the Gordon Types II and IV as "para."

serums were prepared in the laboratory from selected strains, the identity of which had been determined beyond dispute, and in addition to these serums standard English serums and bacterial suspensions were obtained from the Medical Research Committee of London. Some of the serums had been prepared in July, 1917, and were not used until January, 1918, and gave very weak reactions with the bacterial suspensions that had been sent with them from England. Many of the stock meningococcus cultures failed to agglutinate well with the English serums. Some, however, were found to agglutinate with the different types. Many of the cultures that agglutinate with Type III serum gave weak reactions with Type IV serum also. The cultures that gave reactions with the English monovalent type serum were then tested with the polyvalent horse serum to determine if they were agglutinated in dilutions of 1 to 800 or more.

Amoss at the Rockefeller Institute had been using Nos. 1, 7, 10, 30 and 60 in testing commercial serums, and Colonel Whitmore in Washington, D. C., was using Nos. 1, 10, 30 and 60 in the preparation of vaccines. It seemed, therefore, advisable to use as many of these cultures as possible for standards. Culture 1, classed in this country as normal, and by the British Army as Type II, did not agglutinate in the polyvalent horse serum diluted 1 to 800 (and also failed to develop monovalent serums of high potency in the rabbit), so another culture belonging to this group had to be chosen. No. 46 was found to fulfill the requirements. No. 10 and No. 30 were chosen as representative of the irregular group. No. 60 was chosen as representative of the para or British Army Type I. All of these cultures gave a high agglutinating titer in the polyvalent horse serums, and in the rabbits produced monovalent serums of high titer.

Absorption tests were made before these cultures were finally chosen. Monovalent rabbit serums had been prepared against several cultures. Serum "A" against Culture 1, a normal strain. This serum diluted 1 to 400 agglutinated British Army Type II suspension; and diluted 1 to 100 British Army Type IV suspension. Serum "B" against Culture 30, an irregular strain, diluted 1 to 400 agglutinated British Army Type III suspension; diluted 1 to 300 agglutinated British Army Type IV suspension, and diluted 1 to 100 agglutinated British Army Type I suspension. Serum "C" against Culture 60, a para strain, diluted 1 to 300 agglutinated British Army Type I suspension. These three serums represent the three groups as classified in this country. Culture 46 absorbed the agglutinin from the monovalent normal serum "A"; Cultures 10 and 30 absorbed the agglutinin from the irregular serum "B," and Culture 60 absorbed the agglutinin from the para serum "C."

The cultures used for these agglutination and absorption tests were then plated, colonies picked and replated. The fishings from these last plates were then tested against the monovalent and polyvalent serums to prove that their reactions were the same as the original cultures. Then these subcultures were used for the standard strains to be sent to the manufacturers.

As a result of this work the following rules and regulations were drawn up with the advice and counsel of members of the consulting staff of the State Department of Health and the Public Health Council. They were then submitted to the Commissioner of Health for his approval.

RULES AND REGULATIONS FOR THE TESTING OF THE POTENCY OF ANTIMENINGOCOCCUS SERUM[4]

I. Type of Serum.—Antimeningococcus serum for the treatment of meningitis which is offered for sale in the State of New York shall be prepared by the immunization of animals against at least four standard cultures representative of the principal types of the meningococcus.

II. Minimum Standard of Potency.—The potency of antimeningococcus serum intended for therapeutic use, when tested and compared with the standard serum supplied on request by the Laboratory of the New York State Department of Health, shall have an agglutinative action in a dilution of 1 to 800 with at least the four representatives of the principal types of meningococcus, under conditions in which a standard suspension is employed and the tubes maintained for 16 to 24 hours at 55 C.

III. Standard Cultures.—The standard cultures of meningococcus representative of the principal types may be obtained from the Laboratory of the State Department of Health. They are cultures which have been identified by serological tests and which possess distinctive agglutinative and antigenic properties.

IV. Standard Suspensions.—The standard suspensions for performing the agglutinating tests are so prepared as to correspond in opacity with a standard suspension of barium sulphate containing 3 c.c. of a 1 per cent. barium chlorid solution in 97 c.c. of a 1 per cent. sulphuric acid solution. Such a standard meningococcus suspension contains approximately 2,000 million cocci per cubic centimeter.

V. Method of Testing the Potency of the Serum.—Normal saline solution (0.85 per cent.) should be used as the diluent. After dilution with the serum the final mixture should contain 1,000 million cocci. The standard culture suspensions should be added in agglutination tubes (8 to 10 mm. in diameter), to the diluted serums to be tested, and also similar dilutions of the standard serum. These test mixtures should be incubated at 55 C. for from 16 to 24 hours. The titer thus determined is the maximum dilution of the serum in which definite clumping of the meningococcus in the suspension can be easily detected by the unaided eye. The agglutinative titer which is to be recorded is the potency of the serum.

VI. Physical Properties.—The serum shall be free from all the cellular or solid elements of the blood and should not contain traces of hemoglobin or bile which can be detected by the unaided eye. In order to facilitate inspection, the serum should be put up in containers of colorless transparent glass.

VII. Sterility Tests.—The sterility tests of the final product shall comply with the requirements of the United States Public Health Service.

VIII. Expiration Date.—Expiration date for the sale of the serum shall comply with the requirements of the United States Public Health Service.

4. These rules and regulations are subject to revision from time to time.

A CLINICAL STUDY OF MENINGITIS BASED ON TWO HUNDRED AND FIFTEEN CASES

WILLARD J. STONE, M.D. (TOLEDO, OHIO)
Major, M. C., U. S. Army; Chief of Medical Service
AND
RALPH C. P. TRUITT, M.D. (BALTIMORE)
Major, M. C., U. S. Army
BASE HOSPITAL, FORT RILEY, KAN.

During the period Sept. 23, 1917, to July 15, 1918, there were admitted to the Medical Service of the U. S. Army Base Hospital, Fort Riley, 191 patients with meningococcic meningitis and twenty-four patients with meningitis due to other organisms. It is the purpose of this article to review the important diagnostic features presented by this series and to draw certain conclusions from the methods of treatment employed.

INCIDENCE

During the period, Sept. 23, 1917, to June 3, 1918, the date of admission of the last meningococcic meningitis patient, there were admitted to this hospital 24,406 patients. The incidence of meningococcic meningitis to all hospital admissions was, therefore, 0.77 per cent. These admissions were secured from Camp Funston, which housed the 89th and 92nd Divisions of the Army; the Medical Officers' Training Camp, and the Fort Riley Post. The largest number of patients with meningitis was from the recruits at Camp Funston. These recruits were drawn largely from the states of Kansas, Missouri, Nebraska, Colorado, South Dakota, New Mexico and Arizona. Kansas and Missouri have been regarded as endemic meningitis areas. As will be seen from Table 1, the largest number of patients with meningitis were from the states of Missouri and Kansas. Eighty-nine per cent. of all the patients with the disease were from rural districts. (See graphic Chart 1.)

The epidemic began late in September, 1917, and reached its height in November, gradually declining over the following six months. Table 2 shows the admissions by months for meningococcic meningitis. The mortality rate is also shown by months. In 191 cases the mortality was 28.8 per cent.

It will be observed in Table 3 that mixed infection occurred in thirteen instances with mortality of 92.3 per cent., while in Table 4 it will be seen that meningitis due to other infection than meningococcus occurred in eleven instances with mortality of 81.8 per cent.

TABLE 1.—MENINGOCOCCUS MENINGITIS EPIDEMIOLOGY BY STATES *

	Recovered		Deaths		Rural per Cent. Cases
	Urban	Rural	Urban	Rural	
Kansas	4	23	1	16	88.6
Missouri	11	38	0	19	83.8
Nebraska	1	15	0	2	94.4
Colorado	0	8	1	1	90.0
South Dakota	0	8	0	1	100.0
New Mexico	0	0	0	1	100.0
Arizona	0	1	1	1	66.0
Total	16	93	3	41	89.0

* The seven states listed in Table 1 supplied to a large extent the selective draft recruits of the Army, Camp Funston.

TABLE 2.—MENINGOCOCCUS MENINGITIS

Admissions	Recovery	Deaths	Mortality, per Cent.
September	1	1	50.0
October	10	6	37.5
November	43	29	40.0
December	30	7*	18.9
January	16	3	15.7
February	12	3	20.0
March	10	2	16.6
April	5	2	28.5
May	8	1	11.1
June	1†	1	50.0
Total	136	55	Aver. 28.8

* Including one case of lobar pneumonia.
† Clinically abortive type, no organisms found.

TABLE 3.—MORTALITY IN MENINGITIS DUE TO MIXED INFECTIONS

Date	Infection	Recovered	Deaths	Remarks
1917				
Oct.	Streptococcus and meningococcus	1	1	
Nov.	Streptococcus and meningococcus	0	2	
	Streptococcus and pneumococcus	0	1	
	Pneumococcus and meningococcus	0	1	
Dec.	Streptococcus and meningococcus	0	1	
1918				
Jan.	Streptococcus and meningococcus	0	1	Bilateral suppurative otitis media and parenchymatous nephritis
Feb.	Pneumococcus and meningococcus	0	1	
Mar.	Streptococcus and meningococcus	0	2	Both deaths followed postmastoid operation (Strept.) and meningococcus meningitis
May	Streptococcus and pneumococcus	0	2	
	Total	1	12	Mortality, 92.3 per Cent.

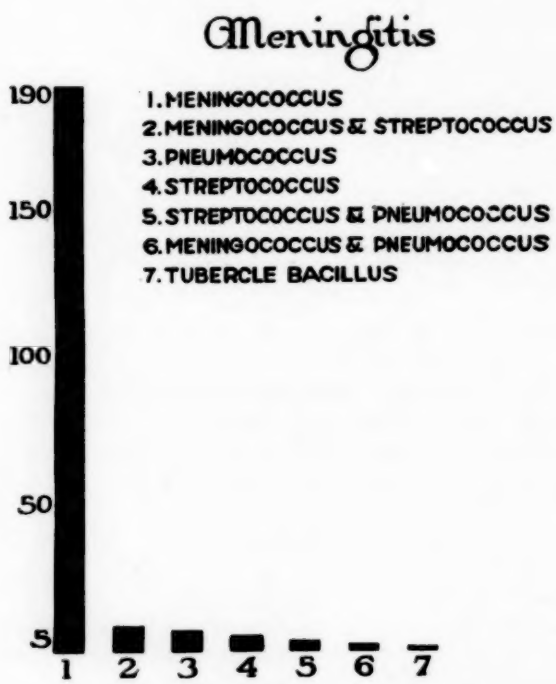

Chart 1.—Types of infection in meningitis.

TABLE 4.—Mortality in Meningitis Due to Other Infections (Not Meningococcus)

Date	Infection	Recovered	Deaths	Remarks
1917				
Oct.	Pneumococcus.....	0	1	
Nov.	Streptococcus.....	0	1	
	Pneumococcus.....	2	0	
Dec.	Tubercle bacillus.....	0	1	
1918				
Jan.	Pneumococcus.....	0	1	Lobar pneumonia with pneumococcic meningitis
May	Streptococcus.....	0	2	Both deaths followed mastoidectomy and streptococcus meningitis
June	Streptococcus hemolyticus	0	1	Skull fracture followed by streptococcus meningitis
July	Pneumococcus.....	0	2	One death due to Type I pneumococcus meningitis during pneumonia; one death due to Type I pneumococcus meningitis and suppurative mastoiditis
	Total.....	2	9	Mortality, 81.8 per Cent.

TABLE 5.—Race Mortality

	Cases Recovered	Fatal Cases	Total Cases		Mortality, per Cent.
Meningococcus:					
White	123	52	175		29.7
Colored	13	3		16	18.7
Mixed infections (Strept. and meningococcus):					
White	1	11	12		
Colored	0	1		1	
Other infections:					
White	2	9	11		
Colored	0	0		0	
All types meningitis:					
White			198		31.3
Colored				17	23.5

Total white, 198 or 92.1 per cent.; total colored, 17 or 7.9 per cent.; total, 215.

TABLE 6.—Epidemiology, Camp Funston, Meningococcus Meningitis

	Recovered	Deaths	Mortality, per Cent.	Remarks
89th Division:				
354th Infantry	19	9	32.1	Includes one recovery from streptococcus and meningococcus meningitis; includes one death from streptococcus and meningococcus meningitis
356th Infantry	14	11	44.0	
353d Infantry	14	6	30.0	Includes one death from streptococcus and meningococcus meningitis
355th Infantry	10	3	23.0	
341st Field Artillery	7	0	0	
340th Machine Gun Bn.	4	2	33.3	Includes one death from streptococcus and meningococcus meningitis; includes one death from pneumococcus and meningococcus meningitis
314th Supply Train	0	1	100.0	
314th Engineers	3	2	40.0	
340th Field Artillery	3	2	40.0	
342d Field Artillery	1	3	75.0	
342d Machine Gun Bn.	2	2	50.0	
341st Machine Gun Bn.	3	0	0	
314th Sanitary Train	3	0	0	
314th Trench Mortar Bn.	1	1	50.0	Includes one death from streptococcus and meningococcus meningitis
314th Ammunition Train	1	1	50.0	
314th Field Signal Bn.	2	0	0	
Quartermaster Corps	1	0	0	
Independent Organizations:				
164th Depot Brigade	19	10	38.0	Includes one death from streptococcus and meningococcus meningitis
323d Field Signal Bn.	0	1	100.0	Includes one death following mastoidectomy with streptococcus and meningococcus meningitis
529th Engineers Service Battalion (colored)	2	0	0	
530th Engineers Service Battalion (colored)	1	1	50.0	
Total	110	55	33.3*	
92d Division:†				
317th Ammunition Train	3	1	25.0	
317th Supply Train	2	0	0	
317th Sanitary Train	2	0	0	
349th Machine Gun Bn.	1	1	50.0	
Total	8	2	20.0	

* Including six deaths mentioned due to mixed infections, streptococcus and meningococcus meningitis and one death due to pneumococcus and meningococcus meningitis.

† The 92d Division was made up of negro troops, largely from the Southern states. The command varied from approximately 12,000 (in October, 1917) to 15,000 men.

Ninety-two per cent. of the patients were white, while 8 per cent. were colored. In 175 white patients the mortality in meningococcus meningitis was 29.7 per cent.; among 16 colored patients the mortality was 18.7 per cent. The same difference in mortality will be noticed in meningitis due to all types of infection, shown in Table 5. For all types of meningitis among 198 white patients the mortality was 31.3 per cent., while among 17 colored patients the mortality was 23.5 per cent.

EPIDEMIOLOGY

The instances of meningitis arising at Camp Funston did not appear to arise from any particular endemic focus, but were more or less widespread throughout the various organizations.

In all, 175 cases were admitted to the hospital from Camp Funston; 13 cases were admitted from the Medical Officers' Training Camp at Fort Riley; 6 cases from the Fort Riley Post; 2 cases from the Base Hospital personnel; while 3 patients were civilians.

TABLE 7.—EPIDEMIOLOGY (MENINGOCOCCUS MENINGITIS) AT FORT RILEY

	Number Recovered	Number Deaths	Mortality, per Cent.
Medical Officers' Training Camp	10	3*	23.0
Post:			
311th Cavalry	0	1	100.0
13th Cavalry	0	1	100.0
40th Infantry	1	0	0
Mounted Service School	1	0	0
Quartermaster Corps	2	0	0
Total	4	2	33.3
Base Hospital:			
Medical Detachment	1	0	0
Army Nurse Corps	1	0	0
Total	2	0	0
Civilians	3	2	40.0

* Includes one death from postmastoid streptococcus (?) and meningococcus meningitis. Includes one death from postlobar pneumonia, pneumococcus and meningococcus meningitis.

TABLE 8.—AVERAGE STRENGTH OF COMMAND, 89TH DIVISION, CAMP FUNSTON, AND INCIDENCE BY MONTHS OF MENINGOCOCCUS MENINGITIS

	Strength of Command*	Incidence per 1,000
October	41,413	0.39
November	28,543	2.57
December	25,973	1.32
January	24,248	0.62
February	23,346	0.65
March	22,414	0.45
April	25,279	0.28
May	29,535	0.31
Average	27,594	0.82

* Based on weekly telegraph reports, Oct. 12, 1917 to June 7, 1918.

The strength of command 89th Division, Camp Funston, varied from approximately 41,000 men in October to 22,000 men in March. The strength of command, 92nd Division (Colored) Camp Funston, varied from 12,000 to 15,000 men. Table 8 shows the incidence of the disease per thousand by months. The highest incidence during the course of the epidemic was 2.57 per thousand for the 89th Division, and 0.26 per thousand for the 92nd Division.

TABLE 9.—APPROXIMATE STRENGTH OF COMMAND, 92D DIVISION, CAMP FUNSTON, AND INCIDENCE BY MONTHS OF MENINGOCOCCIC MENINGITIS

1917	Strength of Command	Incidence per 1,000
October	12,000	0.00
November	15,000	0.00
December	15,000	0.26
1918		
January	15,000	0.26
February	15,000	0.06
March	15,000	0.06

THE "CARRIER" PROBLEM

The first patient with meningococcus meningitis, a civilian laborer, was admitted Sept. 23, 1917. During the week beginning Oct. 16, 1917, sixteen patients were admitted. Since the isolation of carriers was believed to be the best means of combating the spread of the infection, all contacts were cultured by the laboratory staff. We are indebted to Major O. F. Broman, M.C., Chief of Laboratory Service, for the following data covering the enormous amount of work completed by the laboratory staff in the investigation of the problem.

The first 2,557 cultures from contacts revealed 2.5 per cent. carriers. As the work progressed it became apparent that the demonstration of infection, transmitted from patients with the disease to contacts, or from carriers to contacts, was difficult if not impossible, because of the widespread distribution of the infection. It was then determined to culture the entire command at Camp Funston and the Medical Officers' Training Camp at Fort Riley. This was continued as new recruits arrived, with the result that every soldier had repeated cultures. Table 10 shows the number of cultures taken by weeks, the percentage of carriers and the number of patients admitted.

During the period of nine months over 195,900 cultures were taken. It will be observed from the table that approximately 2 per cent. showed positive cultures for meningococci for the entire series. During three different weeks the percentage of positive cultures was over 5 per cent.: from October 29 to November 5 with eighteen meningitis admissions; from November 19 to November 26 with sixteen admissions, and from January 21 to January 28 with six admissions.

For short intervals, in small groups, the number of carriers would approximate 20 per cent., but the highest average for any week was 6 per cent.

TABLE 10.—MENINGITIS CARRIERS

Date	Cultures	Carriers	Per Cent. Carriers	Cases
September 23 to September 30	0	0	0	2
October 15 to October 29	2,557	64	2.5	16
October 29 to November 5	1,746	105	6.0	18
November 5 to November 12	1,437	57	3.9	17
November 12 to November 19	3,044	78	2.5	22
November 19 to November 26	3,030	175	5.7	16
November 26 to December 3	7,343	120	1.6	10
December 3 to December 10	7,478	313	4.2	8
December 10 to December 17	10,674	275	2.6	7
December 17 to December 24	12,011	249	2.0	8
December 24 to December 31	7,939	321	4.0	9
December 31 to January 7	9,185	302	3.2	1
January 7 to January 14	12,814	285	2.2	6
January 14 to January 21	12,271	262	2.1	6
January 21 to January 28	10,653	596	5.6	6
January 28 to February 4	8,813	359	4.0	6
February 4 to February 11	11,434	226	1.8	4
February 11 to February 18	4,931	39	0.8	2
February 18 to February 25	1,207	67	0.5	4
February 25 to March 4	5,502	134	2.4	3
March 4 to March 11	3,263	43	1.3	4
March 11 to March 18	4,074	36	0.8	3
March 18 to March 25	4,748	22	0.5	3
March 25 to April 1	5,126	14	0.2	2
April 1 to April 8	3,718	12	0.3	2
April 8 to April 15	6,197	19	0.3	3
April 15 to April 22	1,572	3	0.2	1
April 22 to April 29	2,096	2	0.1	1
April 29 to May 6	5,352	9	0.2	3
May 6 to May 13	4,141	4	0.09	2
May 13 to May 20	263	0	0.0	2
May 20 to May 27	1,768	2	0.1	1
May 27 to June 3	3,377	2	0.05	2
June 3 to June 10	3,828	2	0.05	0
June 10 to June 17	47	0	...	1*
June 17 to June 24	691	0	...	0
June 24 to July 1	5,091	0	...	0
July 1 to July 8	4,294	0	...	0
July 8 to July 15	5,199	0	...	0
Total	195,904	4,197	2.1	201†

* Abortive type, no organisms found.
† Includes 10 mixed infections with meningococcus listed in Table 3.

It will be observed in Table 11 that the percentage of carriers showed little variation during the months of October, November, December and January, and yet there were about three and one-half times the number of admissions for meningitis in November as in January. Such facts, while admitting various interpretations, leave open to serious doubt certain established theories that the "carrier" markedly influences, except in a sporadic manner to susceptible individuals, the spread of epidemic meningitis. (See Graphic Chart 2.)

It is believed that individuals during the course of an epidemic in a military organization, who are known by repeated cultures to be chronic carriers should be isolated in a detention camp, there to be kept in the open air so far as possible and to receive treatment in the way of mild nasopharyngeal sprays and gargles. A 2 per cent. silver nucleinate nasal spray administered three times daily is slightly

astringent and irritating to the mucous membrane and causes a more or less copious flow of secretion which mechanically assists in the removal of infection. A nasal spray of warm physiologic sodium chlorid solution is likewise efficient. A gargle of Liquor Sodii Boratis Compositus (N. F.) to which has been added 1 drop of phenol to the ounce is likewise useful. A week of such treatment is probably as efficient as a longer period.

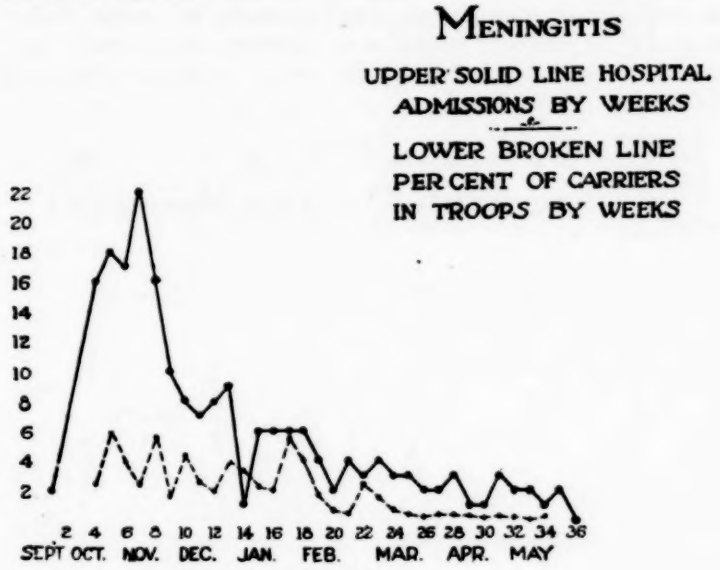

Chart 2

Since a very large percentage of all individuals are immune it is extremely doubtful if any considerable number of carriers can be detected early enough during the beginning of an epidemic greatly to influence its spread. Those who are specifically susceptible will undoubtedly acquire the infection when exposed directly to virulent organisms by sneezing or coughing carriers.

TABLE 11.—PERCENTAGE OF CARRIERS AND ADMISSIONS BY MONTHS COMPARED

	Average Carriers, per Cent.	Number of Meningitis Admissions
October 15 to November 5	4.25	34
November 5 to December 3	3.42	65
December 3 to December 31	3.20	32
December 31 to January 28	3.27	19

In the consideration of the carrier problem it is believed that a carrier is not necessarily a menace to other individuals unless it can be proved that he harbors virulent organisms. Two methods of transmission of the disease may occur. In the one avirulent organisms are transmitted to an individual who is specifically susceptible. Such an individual may or may not develop manifestations of the disease, depending on a variety of factors, such as fatigue accompanied by poor food and lack of sleep, or the presence or absence of other secondary or coincident infections present in an ordinary coryza, which might temporarily exalt virulence. In the other, virulent organisms are

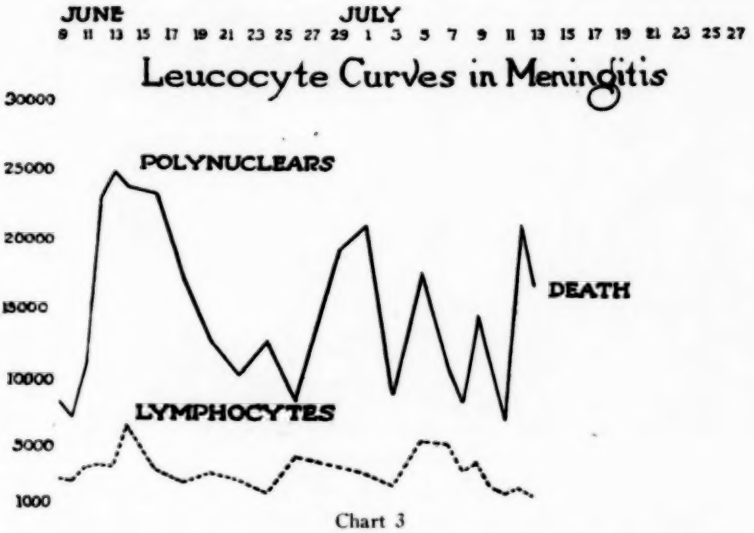

Chart 3

transmitted to an individual with specific susceptibility, but whose immunity temporarily is such that he may for weeks manifest no disease and eventually rid himself of the infection harbored in his nasopharynx. Or he may become hypersusceptible through a variety of inimical influences and the disease become evident.

It is believed that culturing of contacts known to have been more or less closely associated with individuals who manifest the disease should be carried out. This refers in a military organization to close bed associates in the barracks or close table associates in the mess halls. Contacts who show positive cultures should be isolated for at least one week and receive the simple treatment described. Such patients should be kept in the fresh air and sunlight as much as possible.

IMMUNITY IN MENINGITIS

In the series embraced by this study, ten patients out of 191 patients with meningococcic meningitis, or 5 per cent., gave a history of an earlier carrier state. Of these ten patients, eight recovered and two died. It was not possible, because of the stress of work, to take nasopharyngeal cultures of all meningitis patients on admission, but during a series of cultures at different intervals it was found that approximately 10 per cent. of the cultures were positive.

A definite history of earlier meningitis was given by two patients, one of whom recovered while the other resulted fatally. Two patients were readmitted with meningitis several weeks after their recovery

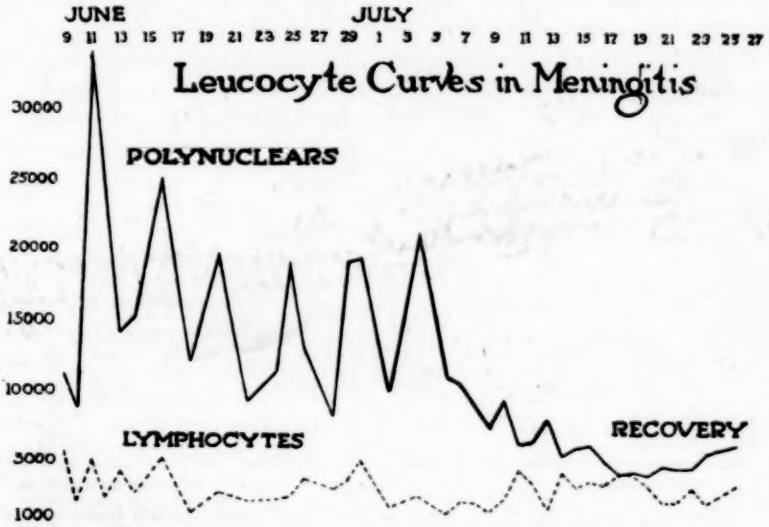

Chart 4

from the first attack. The first patient was in the hospital for thirty-two days with a typical moderately severe attack. Sixty-five days after his discharge from the hospital he was readmitted unconscious in a second attack, from which he recovered in thirty-three days. In a little over four months he had suffered two attacks of meningitis. The second patient was in the hospital forty-two days with a moderately severe meningitis. Eighty-four days after his discharge he was readmitted in a typical attack. He was sensitized to serum from the earlier intraspinal injections for, despite attempts at desensitization. the injection on the first three days of small quantities of serum intravenously was followed by severe anaphylactic reactions. This patient

presented an added difficulty in the way of treatment, since ventricular or spinal blockage had occurred from the earlier attack, and it was impossible to secure enough spinal drainage to give injections of serum into the canal. Severe headache, high fever and mild delirium occurred at irregular intervals. Injections of sensitized vaccine were begun in doses of from 500 to 2,000 millions every alternate or third day, with marked improvement in his condition. In all about twelve injections were given. He was discharged recovered four months after the onset of his second attack.

SYMPTOMS

The disease manifested itself, in most instances, by the sudden onset of headache, stiff neck, nausea or vomiting and chills, followed in a few hours by delirium and coma. So rapid was the onset in many instances that patients who complained of slight headache and nausea at bedtime were found unconscious in their beds the following morning. Table 12 shows the subjective initial symptoms in 136 recovered and fifty-five fatal instances of the disease.

TABLE 12.—INITIAL SYMPTOMS

Subjective Initial Symptoms in 136 Recovered Instances*		Subjective Initial Symptoms in 55 Fatal Instances*	
Symptoms	Per Cent.	Symptoms	Per Cent.
1. Headache	85	1. Stiff neck	46
2. Stiff neck	72	2. Nausea and vomiting	32
3. Nausea and vomiting	70	3. Headache	30
4. Chills	41	4. Fever	22
5. Fever	32	5. Chills	16
6. Sore throat	12	6. Backache	1.3
7. Backache	8	7. Coryza	1.3

* It is highly probable that the actual percentages of many of these symptoms should be higher but were not recorded on examination. For practical purposes they represent the relative sequence of subjective symptoms. In addition, photophobia should be mentioned as an especially suggestive symptom.

Fever was associated with chills or chilly sensations in one-third of the cases, while an inflamed pharynx, backache or coryza was mentioned less frequently.

Objectively, on admission, Kernig's sign was mentioned on the clinical history in 67 per cent. of 136 recovered instances. It is highly probable that in many cases this sign, as well as other symptoms, were omitted from the clinical record due to the stress of work, although found to be present at the time of examination. Kernig's sign has been in our experience almost universally present in meningitis, if not during the first twelve hours it is usually easily demonstrated at some time during the first twenty-four or thirty hours after the onset of

headache and neck rigidity. Stupor and coma were present in 51 per cent. of the recovered patients on admission, delirium in 29 per cent., exaggerated reflexes were mentioned in 23 per cent., dilated pupils in 22 per cent., and herpes labialis in 8 per cent. Petechiae were evident in only 6 per cent. of 136 recovered patients, but were present in 14 per cent. of fifty-five fatal instances. A leukocytosis varying from 15,000 to 35,000 was an important early symptom.

TABLE 13.—Objective Initial Symptoms

Objective Initial Symptoms in 136 Recovered Instances of Meningitis		Objective Initial Symptoms in 55 Fatal Instances of Meningitis	
Symptoms	Per Cent.	Symptoms	Per Cent.
1. Kernig's sign	67	1. Stupor and coma	42.6
2. Stupor and coma	51	2. Delirium	40.0
3. Delirium	29	3. Kernig's sign	37.0
4. Exaggerated reflexes	23	4. Dilated pupils	21.0
5. Dilated pupils	22	5. Petechiae	14.0
6. Babinski's sign	15	6. Retention urine	13.3
7. Herpes labialis	8	7. Exaggerated reflexes	9.3
8. Retention urine	6.7	8. Babinski's sign	8.0
9. Strabismus	6	9. Local convulsive movements	5.3
10. Petechiae	6	10. Recent mastoidectomy	5.3

ABORTIVE TYPES OF MENINGITIS

Abortive types of epidemic meningitis are not uncommon and present great difficulties in proper recognition and care. We have seen many patients who presented during the course of an epidemic symptoms of the disease, headache, fever, vomiting, rigid neck, photophobia and Kernig's sign, but in whose spinal fluid meningococci could not be found. The fluid in such cases was under increased pressure and contained a moderate number of polymorphonuclear leukocytes. In searching for organisms in such cases the fluid should be centrifuged at high speed and 1 c.c. from the bottom of the tube allowed to dry on a glass slide before staining. The blood culture has been sterile in these patients.

In such abortive types with marked clinical evidences of the disease, especially in the presence of an epidemic, serum should be administered after free drainage at the time of diagnostic puncture. The clinical evidences in such instances markedly decreased after twenty-four hours, and recovery was complete in from seven to ten days. The recognition of such abortive types has been difficult in some patients because of the possibility of meningismus associated with a beginning pneumonia, the presence of acute cerebrospinal syphilis, of early tuberculous meningitis or of catalepsy or hysterical states associated with fever.

COMPLICATIONS

The serum rash and arthritis which occurred in about 30 per cent. of the recovered cases were of transitory duration as a rule. As mentioned in Table 14, in only one instance did culture from aspirated joints show meningococci. Temporary deafness occurred in about 7 per cent. of the recovered patients. Permanent nerve deafness, bilateral, occurred in 3.6 per cent. It came on early and suddenly and was the most distressing and disabling complication. Mixed infection was the most serious complication as to prognosis.

TABLE 14.—COMPLICATIONS IN 139 RECOVERED INSTANCES OF MENINGITIS

	Per Cent.		Per Cent.
Serum rash	30.0	Adenitis nonsuppurative	2.2
Arthritis	24.0*	Erysipelas	1.4
Parotitis	8.6	Multiple abscesses	1.4
Temporary deafness	7.2	Permanent deafness (partial)	1.4
Orchitis	4.3	Paralysis of arm (temporary)	1.4
Otitis media	4.3	Paralysis of palate (partial)	0.7
Tonsillitis acute follicular	4.3	Paralysis of trunk (partial)	0.7
Permanent deafness (total)	3.6	Paralysis of deltoid	0.7
Corneal ulceration	3.6	Paralysis of tongue	0.7
Rubeola	2.9	Paralysis of peroneus (permanent)	0.7
Cystitis	2.9	Neuritis of extremities	0.7
Lobar pneumonia	2.2	Phlebitis	0.7
Adenitis suppurative	2.2†	Panophthalmitis, enucleation of eye‡	0.7

* Arthritis developed in most instances about the time of serum rash and was probably due to the same cause. In only one instance did culture from aspirated joints show meningococci.

† Due to streptococcus infection.

‡ Cultures from vitreous humor revealed growth of meningococci.

TABLE 15.—COMPLICATIONS IN 76 FATAL INSTANCES OF MENINGITIS

	Per Cent.
Mixed infection (pneumocdcus or streptococcus with meningococcus)	15.8
Serum rash	4.0
Otitis media	4.0
Lobar pneumonia	4.0
Lobar pneumonia with pneumococcic meningitis	2.6
Mastoiditis suppurative with Type I pneumococcic meningitis	1.3
Mastoiditis with streptococcic meningitis	2.6
Skull fracture with streptococcic meningitis	1.3
Fracture of ileum and peritonitis	1.3
Streptococcic peritonitis (cause not found)	1.3
Inanition	1.3
Nephritis acute	1.3
Optic neuritis	1.3
Facial paralysis	1.3

PATHOLOGY

Complete records of twenty-six necropsies were available in this series. In the epidemic form the following conditions were usually found. The brain was irregularly covered with a fibrinopurulent exudate. This exudate was most marked along the course of the larger blood vessels of the cortex, about the points of exit of the cranial nerves and at the base over the pons and medulla. Usually,

marked edema of the brain was present, with obliteration of the sulci of the cortex. The pia-arachnoid vessels of the cortex were markedly injected. Punctiform hemorrhages were frequently noted in the ventricular spaces, which were dilated and contained cloudy fluid. The cavities of the third and fourth ventricles frequently contained a fibrinopurulent exudate which covered the choroid plexus. Punctiform hemorrhages also were found in the corpus striatum.

In some instances the entire spinal cord was enveloped with exudate. This was usually more marked over the posterior surface and involved the point of exit of spinal nerve roots. Walled-off pockets, with obliteration of the subarachnoid space, were found in some instances, which accounted for difficulties in securing adequate spinal drainage. The exudate was made up of polynuclear leukocytes and fibrinous debris.

Hypostatic pneumonia was frequently found to be present in those patients who had been ill a number of weeks. This was usually associated with edema. Acute or subacute cloudy swelling of the kidneys, liver, spleen and myocardium was usually present. Cultures from the brain and cord in nearly every instance revealed meningococci, while cultures from spleen, heart blood, liver and kidneys were negative.

In septic meningitis due to streptococci or pneumococci, middle ear disease, frequently bilateral, acute mastoiditis, ethmoiditis or empyema of the sinuses was frequently found. In two instances which followed a putrid bronchitis the primary focus of infection was found to have arisen from abscess of the lung. Direct extension of the infection to the meninges from infected mastoid cells prior or subsequent to operation was not common. The organisms present in such cases were usually hemolytic streptococci or pneumococci. In a number of instances of meningitis subsequent to mastoiditis, meningococci were found in the spinal fluid, while the secretion from the mastoid contained streptococci or pneumococci. These patients were probably meningococcic carriers at the time of the onset of the mastoiditis, or were exposed to a carrier after the onset of the mastoid infection.

TREATMENT

Lumbar puncture was performed for diagnostic purposes as early as possible on all meningitis suspects, either in the Receiving Ward where the patients were isolated in cubicles, or in a separate cubicled suspect ward adjacent to the meningitis section. If the fluid was under considerable pressure, drainage was allowed to proceed until the pressure approximated the normal, one drop every three or four seconds. If the fluid was cloudy, an injection of 30 c.c. of serum was given without waiting for the laboratory report. In some instances the

fluid was under increased pressure but was clear, in which case, if the clinical symptoms were sufficiently suggestive, an injection of serum was given at the time of diagnostic puncture.

The fluid in streptococcus or mixed infection meningitis, as a rule, had a straw-yellow tinge, while the fluid of meningococcus meningitis had a grayish-white opaque appearance. After several intraspinal injections of serum had been given, the spinal fluid became slightly yellowish in the treated patients. In numerous instances despite great care on the part of the officers in the laboratory especially detailed for the examination of spinal fluids, no organisms would be found in the centrifugalized fluid obtained from the first and, in some instances, the second punctures. Again, in a series of punctures organisms would be found on one occasion only. As a rule, however, there was little difficulty in finding the organisms in the fluid obtained by the daily puncture. A daily specimen of fluid was sent to the laboratory from each patient. In 90 per cent. of 215 instances the spinal fluid was distinctly cloudy on first puncture.

TABLE 16.—The Spinal Fluid in Meningitis, First Puncture

Recovered 139	Per Cent.	Fatal 76	Per Cent.
1. Cloudy	91.3	1. Cloudy	88.1
2. Clear but with leukocytes microscopically and positive globulin	6.4	2. Clear but with leukocytes microscopically and positive globulin	9.2
3. Clear with no leukocytes or globulin	1.4	3. Clear but organisms found	2.6
4. Clear but organisms found	0.7		

The usual amount of serum given intraspinally was from 30 to 35 c.c. twice daily during the first five or six days until the reports received from the laboratory showed two or more negative examinations. The amount of drainage of spinal fluid depended on its pressure, whether headache became more marked during the procedure or whether, as happened in many instances, relief of headache and restlessness was secured by it. As a rule, the quantity of fluid drained exceeded by from 15 to 25 c.c. the quantity of serum to be given. The average amount of serum drained was approximately 50 c.c., but in a number of instances quantities varying from 70 to 85 c.c. were obtained without markedly decreasing the pressure, in which case larger quantities were secured. In Table 17 it will be noticed that the average number of intraspinal treatments in 136 recovered patients was eleven; the average total amount of serum received by each was 305 c.c., the average total drainage of spinal fluid was 571 c.c. The average stay in the meningitis section among these recovered patients was fifty-two days.

Among the fifty-five fatal instances of meningococcic meningitis the average number of intraspinal injections was ten; the average total amount of serum given was 310 c.c., while the average total amount of fluid drained was 558 c.c. The average number of days under treatment in these fatal instances was 10.5.

TABLE 17.—DATA OF TREATMENT

	Meningococcus Meningitis		Mixed Infections	
	Recovered	Fatal	Recovered	Fatal
Number of patients	136	55	1	12
Average number I. S. injections	11	10	12	6
Average total amount serum, c.c.	305	310	415	151*
Average total spinal drainage, c.c.	571	558	475	251
Days in hospital	52	10.5	147	9

* Antistreptococcus, antimeningococcus or antipneumococcus serum.

INTRAVENOUS THERAPY

Much interest has followed the treatment recently advocated by Major Herrick[1] and by Netter[2] of combined intravenous and intraspinal therapy in meningitis. In a large percentage of the cases reported by Herrick from Camp Jackson, South Carolina, petechiae were common during the initial stage of the disease. This led to the belief that early in the course of the disease the blood stream was infected, followed later by localization in the meninges. This logical assumption is apparently true for certain types of meningococcus infection, but that it is true for all types is not so easily proved. In this series but 6 per cent. of the recovered patients showed petechiae. Among the fatal instances, 14 per cent. showed petechiae. Included among these were the rapidly fulminating types with death in a few hours. In some of these the cerebrospinal evidences of the disease were found to be slight on necropsy, although the meningococcus was obtained by culture. The evidence pointed to early blood stream invasion with death from sepsis. The following abstracts of clinical histories illustrate this point:

CASE 1.—April 11, the patient was admitted to the hospital with transfer diagnosis of influenza. He complained of headache. In a few hours a syncopal attack occurred, with involuntary micturition, followed by transitory delirium and muscular contractures in the extremities. A stuporous condition soon supervened. Lumbar puncture showed only a few leukocytes; no organisms. Kernig's sign was positive. General adenitis was present, but his neck was only slightly rigid. The second lumbar puncture did not reveal meningococci, but 40 c.c. of antimeningitis serum were given intraspinally and 60 c.c. intravenously. Death occurred in about twenty-four hours from time of onset. Large hemorrhagic spots the size of the hand appeared over both ankles and on the face, with smaller petechiae scattered over the trunk about six hours before death. These spots coalesced until at time of death the body was covered with confluent areas, with but small areas of white skin remaining. At

1. J. A. M. A. **71**:612, 1918.
2. Rev. de méd. **35**:133, 1917.

necropsy there was no gross evidence of meningitis, but cultures of small suspicious foci showed meningococci. The nasopharyngeal culture and the blood culture showed meningococci.

Case 2.—The patient, a civilian, previously well, was suddenly taken with chill, nausea and vomiting. His general condition became rapidly worse during the following eleven hours; the extremities became cyanotic and large hemorrhagic spots appeared over the body. Three hours later he was admitted to the hospital; temperature 104, pulse 170, barely perceptible at the wrist; respiration was embarrassed and he was stuporous. The spinal fluid was clear, with but few cells. A few extracellular meningococci were found. Two intraspinal injections of serum were given (80 c.c.) and three intravenous injections (140 c.c.) during the following eighteen hours. Death occurred in thirty-two hours after onset. The blood culture showed meningococci.

TABLE 18.—Combined Intraspinal and Intravenous Therapy in Meningococcus Meningitis

Recovered				Died				
Number Injections, Intravenous	Total Amount Serum, C.c.	Number Injections, Intraspinous	Total Amount Serum, C.c.	Number Injections, Intravenous	Total Amount Serum, C.c.	Number Injections, Intraspinous	Total Amount Serum, C.c.	Days Under Treatment Before Death
1	20	10	250	18	730	20	460	29
1	100	7	140	3	140	2	80	1
9	300	15	380	11	420	9	340	17
4	140	9	255	1	80	13	240	12
6	140	13	320	1	20	3	60	2½
5	100	8	200	10	300	35	1,080	30
9	190	11	200	1	60	1	40	1
11	220	17	380	2	40	10	255	6
10	380	13	310	4	200	40	1,015	41
11	320	13	200					
8	210	17	395					
5	100	25	555					
3	60	0	0*					
2	40	10	275					
3	70	9	225					
3	60	17	460					
4	75	0	285					
4	75	13	365					
4	80	7	240					
3	60	8	240					
2	40	0	250					
2	35	7	230					
4	170	31	795					

Recovered:	
Average number intravenous injections.....	5
Average total amount serum received.....	130 c.c.
Average number intraspinal injections.....	13
Average amount serum received.....	329 c.c.
Total patients, 23	
Deaths:	
Average number intravenous injections.....	5
Average total amount serum received.....	228 c.c.
Average number intraspinal injections.....	15
Average total amount serum received.....	397 c.c.
Total patients, 9	

Mortality in 32 instances having combined treatment, 28.1 per cent.
Twenty-six patients received more than 50 c.c. serum intravenously, while seventeen patients received 100 c.c. or more.
* Readmitted patient with ventricular or canal blockage from earlier attack, in whom it was impossible to give serum intraspinally.

Table 18 describes the use of combined therapy in thirty-two patients. The mortality was 28.1 per cent. Three of the patients died within sixty hours after admission. In one of the fatal cases the patient received 730 c.c. of serum intravenously in eighteen injections, and 460 c.c. of serum intraspinally in twenty injections; in two other fatal cases the patients received 200 and 360 c.c., respectively, by vein and 1,080 and 1,015 c.c., respectively, into the spinal canal. The mortality in this series of patients was almost identical with the mortality among 159 patients with meningococcic meningitis treated intraspinally (deaths forty-six; mortality 28.9 per cent.). The criticism

may be offered that enough serum was not given by vein to some of these patients. To this it may be replied that as much was given as appeared advisable at the time, because of the occurrence of symptoms of anaphylaxis.

We feel that patients manifesting petechiae, as evidence of blood stream invasion, should receive intravenous injections of serum in doses of from 60 to 100 c.c. daily during the first three or four days of the disease, as well as intraspinal injections varying from 30 to 40 c.c. of serum twice daily. If success is to be secured, early energetic treatment is essential, and is of greatest value during the first three or four days. Although we believe that the blood stream infection is transitory, with relatively early localization in the meninges, the method of combined intravenous and intraspinal therapy should receive thorough trial.

REMARKS ON TREATMENT

During the course of treatment in a number of instances the spinal fluid became decreased in quantity, which made it exceeding difficult to administer intraspinally a sufficient amount of serum without danger of increased pressure. In such cases it was assumed that ventricular or canal blockage had occurred. If the attempt was made at such a time to give intravenously, after desensitizing doses of 0.5 c.c. subcutaneously, followed in an hour by 1.0 c.c., chills and collapse, symptoms attributed to anaphylaxis, occurred. Cyanosis with rapid pulse and dyspnea followed in some instances. These symptoms, although alarming, were usually alleviated in from ten to twenty minutes by an injection subcutaneously of 10 or 15 minims of 1 to 1,000 solution epinephrin chlorid. If the dosage by vein was repeated, a hypodermic injection of atropin, 1/100 grain, or of epinephrin prevented the onset of such symptoms.

If the treatment was begun early in the disease by vein as well as by spinal canal the symptoms enumerated rarely occurred; it was only after several days of intraspinal treatment that symptoms occurred if the attempt was made to inject by vein. The method of injection of serum by vein was the same in both instances. The serum was warmed by placing the bottle, connected with a three-way stop-cock and syringe, in a pan of water. The injection of serum was given slowly at a rate of 1 c.c. per minute for the first 15 c.c. If evidences of serum reaction occurred the injection was stopped and the attempt made twelve hours later after a preliminary injection of atropin, 1/100 grain, or epinephrin chlorid solution.

The point is made that in a patient still manifesting evidences of the disease, such as positive organisms or fever, in whom mechanically it becomes difficult to continue treatment by spine, the attempt should

be made to give serum by vein. In a fairly large percentage of such cases serum reactions will occur, but it is believed that the danger of such reactions, especially if atropin or epinephrin is used, and if desensitization has been properly carried out, is infinitely less than the danger of stopping all serum treatment. If it is believed after a number of treatments that little progress is being made, the agglutinative titer of the serum should be determined against pure cultures of the organism. A potent polyvalent serum should agglutinate in dilution 1 to 200, or higher, the usual strains of meningococci, using normal horse serum in dilution of 1 to 100 as a control. If the serum used does not give such agglutination, another make of serum should be tried. It is highly desirable in the presence of an epidemic to determine by agglutination tests the prevailing type of meningococcus encountered. This may be done by the use of monovalent rabbit serum furnished by the Army Medical School against the strains produced by the Rockefeller Institute, namely, Normal No. 1; Intermediate A. No. 10; Intermediate B. No. 30 and Parameningococcus No. 60.

Epinephrin chlorid injections subcutaneously of 15 minims of the 1 to 1,000 solution usually promptly relieved the urticaria which was so commonly seen between the seventh and tenth days of treatment. The administration of morphin was necessary in many instances to relieve restlessness and was believed to be an important adjunct in treatment at any time during the acute stage. For the treatment of herpes labialis alkalinization by soda bicarbonate or the so-called "Imperial Drink"[3] may be recommended. Since herpes labialis has been recognized as associated with acidosis, our patients have been given alkalies routinely with the result that these lesions have been much less evident in the wards. If difficulty is experienced in giving alkalies by mouth, the following may be given by the drop method per rectum: dextrose 30.0; sodium bicarbonate 15.0; tap water 1,000.0.

The question when to stop the treatments by spine has been a difficult one in many instances. The spinal fluid may have contained no organisms after two or three treatments and then a week or ten days later organisms would be found. If the patient had been doing well with but slight elevation of temperature and a decreasing actual and polymorphonuclear differential count, and had had eight or ten treatments, free spinal drainage for a few days was in most instances all that was required. It was not believed, after supposed saturation with serum, that much benefit was secured by further serum injections. We have seen distinct harm produced by overzealous administration

3. Imperial Drink consists of cream of tartar one teaspoonful, sodium citrate and sugar, of each one-half teaspoonful, water flavored with orange or lemon juice, sufficient to make 8 ounces.

of serum after quantities of from 300 to 400 c.c. had been given, solely because the fluid still contained organisms. One patient who had been extremely ill received, after a lapse of a week, an additional treatment by spine because organisms were found in small numbers after a period of negative reports. He became unconscious and remained so for about one week, from which he gradually recovered. We wish to lay stress, from our experience, on the fact that early intensive treatment is necessary to secure results, but we strongly feel that harm may be done by overtreatment after the first ten days or two weeks.

The fever, as a rule, gradually declined after a few treatments. If a remission occurred, associated with positive organisms, it was found advisable to give an additional treatment. The aim has been to be guided by these remissions as a useful index to the frequency of treatment after the first week.

PROGNOSIS

With decrease of fever occurring gradually during the first week, together with return of consciousness and a steady decrease in the actual and polymorphonuclear differential count, made every alternate day, the outlook as to life was good despite the fact that headache and neck rigidity persisted. From the work done by Capt. Edmond Holberg, M.C., in the meningitis wards we are reproducing two charts (3 and 4), which typify the prognostic value to be attached to the actual and differential leukocyte counts in this disease. The prodromal symptoms are important since their recognition leads to early diagnosis and treatment, on which the prognosis depends. These prodromal symptoms of malaise, depression, slight fever and muscular aching are associated with the development of other acute infections. In the presence of an epidemic such symptoms, with headache or nausea, assume great importance, especially if small petechiae are found on the trunk, face, mucous membranes of the mouth or in the conjunctivae. If the disease can be recognized early, and be efficiently treated, the mortality should not be greater than 20 per cent., which would be in marked contrast to statistics covering the period before the adoption of a potent antiserum. The future promises still greater reduction as experience and judgment in its use accumulates, especially in the use of convalescent human serum for the treatment of the acute disease, a plan for which has been worked out in this hospital.

SUMMARY

1. Eighty-nine per cent. of patients with meningitis in the series here reported were from rural districts.

2. The incidence of meningococcus meningitis to hospital admissions in eight and one-half months was 0.77 per cent. The highest

incidence of the disease in any month was 2.57 per thousand white troops and 0.26 per thousand colored troops.

3. The largest number of patients were from the states of Kansas and Missouri, which have for a number of years been recognized as endemic meningitis areas.

4. The mortality in 191 instances of epidemic meningitis was 28.8 per cent.

5. The mortality in thirteen instances of meningitis due to mixed infections, such as streptococcus or pneumococcus with meningococcus, was 92.3 per cent.

6. The mortality in eleven instances of meningitis due to other organisms than the meningococcus was 81.8 per cent.

7. Among 175 white patients with meningococcus meningitis the mortality was 29.7 per cent.; among sixteen negroes the mortality was 18.7 per cent.

8. During the course of the epidemic 196,000 cultures were taken for carriers. The highest percentage of carriers found was 6 per cent.; the average for the entire series 2.1 per cent.

9. Five per cent. of patients with meningitis had been under treatment for the carrier state at an earlier date.

10. The spinal fluid was cloudy in 90 per cent. of first punctures. The fluid was clear, but organisms found, in less than 1 per cent. of the recovered patients and in 2.6 per cent. of the fatal instances.

11. The average number of intraspinal treatments in 136 recovered patients was eleven; the average total amount of serum each received was 305 c.c.; the average total spinal fluid drainage was 571 c.c. The average period of illness was fifty-two days.

12. Among fifty-five fatal instances the average number of days under treatment before death was 10.5.

13. Six per cent. of the recovered patients and 14 per cent. of the fatal instances exhibited petechiae.

14. Positive blood cultures were obtained in a larger percentage of patients with petechiae, while negative blood cultures were as a rule obtained in those patients not manifesting petechiae.

15. The mortality among thirty-two patients who received combined intravenous and intraspinal therapy was 28.1 per cent.

CONCLUSIONS

1. The early diagnosis of abortive types of meningitis, especially during an epidemic, is important.

2. Although recognizing the importance of carriers and their relationship to instances of the acute disease, it is doubtful if carriers can be detected early enough during the course of an epidemic to limit its spread.

3. Intimate contacts, such as tent mates, close bed associates in the barracks and close mess table associates should be cultured upon the development of a case of the disease. If a carrier is found he should be isolated, receive the simple treatment before outlined, and be kept in isolation until four or five negative nasopharyngeal cultures have been returned. These may be taken at two-day intervals.

4. Early energetic treatment is of greatest importance. This embodies, especially in those manifesting petechiae, intravenous and intraspinal therapy. The following plan is recommended: First day, one intravenous injection of from 60 to 80 c.c. serum and two intraspinal injections of from 30 to 40 c.c. each after spinal drainage of from 45 to 55 c.c. of fluid, depending on its pressure; second day, same, except that from 80 to 100 c.c. of serum should be given intravenously, and two spinal injections; third and fourth days, if necessary, repeat; fifth to eighth day, one spinal injection; ninth and tenth days, one spinal drainage daily.

5. It has always proved wise to desensitize before giving the first intravenous injection, by a subcutaneous injection of 1 c.c. of serum. The intravenous injections should be given slowly, at the rate of 1 c.c. of warmed serum per minute for the first 10 or 15 c.c. If anaphylactic symptoms occur the injection should be stopped and the attempt repeated later. Epinephrin chlorid, 1 c.c. of the 1 to 1,000 solution, by hypodermic injection, relieves the symptoms of anaphylaxis. Atropin, grain 1/100, by hypodermic injection is also useful. The serum if mixed, not shaken, with an equal volume of salt solution is less apt to clog the needle.

6. If patients have been given a number of intraspinal treatments and subsequently an intravenous injection is contemplated, extreme care in its administration is necessary to avoid serious symptoms of anaphylaxis.

7. Overtreatment after the first ten or fourteen days may do positive harm.

8. The actual and differential polymorphonuclear leukocyte count is of value in prognosis.

NOTE.—Grateful acknowledgment is made to the following officers for service and suggestions during the course of the epidemic: Majors Andrew MacFarlane and Oscar F. Broman; Captains James W. Osborn, Henry J. Hayes, Robert L. Benson, Edward D. O'Neill, Clark W. Zugg, Edmond A. Holberg and Lieut. James R. Sanford.

PLASMAPHAERESIS IN THE TREATMENT OF CHRONIC NEPHRITIS AND UREMIA*

JAMES P. O'HARE, M.D., HAROLD H. BRITTINGHAM, A.B.,
AND
CECIL K. DRINKER, M.D.
BOSTON

In 1914 Abel, Rowntree and Turner[1] reported on the physiologic effects in dogs of bleeding, removing the plasma and returning the corpuscles after washing with Locke's solution. Such a process was appropriately designated "plasmaphaeresis," and it was shown by these authors and by Turner, Marshall and Lamson[2] that it could be carried to a very considerable extent with entire recovery of the experimental animal. The technic presented in these articles required the use of herudin for prevention of blood coagulation, and positive evidence was secured that the German preparation of herudin then on the market had toxic properties of high order.[3] While the authors in question have offered a number of suggestions as to the possible practical use of plasmaphaeresis, the difficulty of the maneuver, coupled with the impossibility of using herudin, seems to have prevented its clinical employment. In the course of experiments designed to disclose the toxic elements in citrated blood transfusions it became expedient to obtain data on removal and return of corpuscles from the same individual. In consequence of this need the ordinary venesections in uremia patients, which would have been carried through without return of corpuscles, were made instances of plasmaphaeresis, and data were obtained on the possible beneficial effects of this measure. This material is deemed worthy of a brief report.

Technic.—The blood was drawn into sodium citrate, centrifugalized and prepared for return, as indicated in a previous article,[4] except that in no case was sodium citrate added before reinjection, nor was Ringer's solution employed. Cases of chronic nephritis with hypertension are particularly favorable subjects for the necessary technic. It was our practice to withdraw 700 or 800 c.c.

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* From the Medical Clinic of the Peter Bent Brigham Hospital and the Laboratory of Physiology of the Harvard Medical School.

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of blood, centrifugalize, wash twice with freshly made sterile 0.85 per cent. sodium chlorid solution and to return the corpuscles made up to the original volume with salt solution. On completing this return, and without inserting another needle, 700 or 800 c.c. of blood were again withdrawn. The corpuscles thus removed were washed twice and reinjected. The patient never experiences a hemorrhage of more than 800 c.c., and he is subjected to no operation except venepuncture, as it should never be necessary to cut down on a vein in such cases.

The removal of toxic products from 1,500 to 1,700 c.c. of blood cannot result in very large alteration in the patient's plasma. On several occasions we observed rather striking but brief subjective improvement in our patient, but are inclined to attribute it to the psychic effect of the formidable therapeutic maneuver to which the patient was subjected, rather than to any true benefit. The operation has been done eighteen times on eight patients, and the history of one case is presented. Other cases have been so similar as to merit no comment.

REPORT OF CASE

History.—Patient, D. J. McD., man, aged 49, Sept. 17, 1917.

Diagnosis.—Chronic nephritis, chronic uremia, hypertension.

Complaint.—General weakness, dyspnea and cough.

Family and Marital History.—Unimportant.

Habits.—Alcohol in excess.

Occupation.—Steamfitter.

Previous Medical History.—Measles and mumps in infancy. Good recovery and no complications. Weight 210 pounds, four years ago; has lost 53 pounds in the past four years.

Present Illness.—In 1912 the patient first noticed swelling about the ankles, particularly at night, but was not troubled by other symptoms until 1913, when he gradually began to have dyspnea, polyuria, especially nocturia, intense thirst and constant fatigue. Dizziness and violent headaches were soon added, and on one occasion he had an extremely severe nose bleed. By resting frequently the patient has kept at work, but has never felt really well. Vision has grown very bad. For the past twelve days he has been "all in," very dyspneic and drowsy, but unable to sleep. He is entirely unable to lie down on account of a "choking feeling."

Physical Examination.—A well-developed man, sitting upright in bed and showing occasional periods of dyspnea. He appears very ill. Face: Thin, very pale, pasty and expressionless. Eyes: There is external strabismus of the eye with ptosis of the left lid. No photophobia, lacrimation, diplopia, nystagmus, fid-lag or exophthalmos. Conjunctivae pale. The vision of the left eye is partially gone save for light, while that of the right is quite defective and blurred. Ophthalmoscopic examination: Left eye, pupil fixed, media clear, whole fundus pale and dotted with small hemorrhages. Disk covered with exudate and slightly elevated. Right eye, fundus pale. Above the disk there is a small patch of exudate and several hemorrhages. Vessels here, as in the left eye, are tortuous and pulsate slightly.

Thorax: Symmetrical; respirations 28, deep and regular. Heart: Apex impulse forcible in fifth space 12 cm. from midsternal line. No murmurs and no irregularities. Aortic second sound increased. Vessels: Marked sclerosis of radial and temporal arteries.

Blood pressure: Systolic, 225; diastolic, 185.

Lungs: Numerous râles are heard at both bases.

Edema: Moderate in both ankles. No fluid in abdomen or chest.

Further physical findings are unimportant. On admission the patient presented a typical instance of advanced chronic nephritis and hypertension with uremia.

September 17.

Laboratory Report.—Blood: Hemoglobin, 57 per cent. Red blood count, 2,040,000. White blood count, 12,200. Smear: Polymorphonuclears, 80 per cent.; lymphocytes, 15 per cent.; large mononuclears, 3 per cent.; eosinophils, 1 per cent.; mast cells, 1 per cent. Anisocytosis, macrocytosis and moderate achromia were noticed in red cells.

September 18.

Urine: Amber, pale, turbid, acid. Specific gravity, 1.013; large trace of albumin, no sugar. Sediment: occasional white blood corpuscles; large number hyaline, granular and epithelial casts. Phenolsulphonephthalein test, 20 per cent. in two hours (urine volume 175 c.c.).

September 19: The patient is sleepless and often incoherent. His dyspnea has not improved. Owing to the pronounced secondary anemia venesection does not seem advisable and plasmaphaeresis will be employed.

8:33 a. m., 700 c.c. of blood were withdrawn in the usual way into 25 c.c. of 8 per cent. sodium citrate, centrifugalized and red cells washed twice in freshly made sterile 0.85 per cent. sodium chlorid solution, care being taken to discard all buffy material.

12 noon, 500 c.c. saline suspension of these red cells were injected. On finishing this infusion 750 c.c. of blood were withdrawn through the same needle into 25 c.c. of 8 per cent. sodium citrate solution. This blood was treated as in the first case.

2:10 p. m., 550 c.c. saline suspension of washed cells from second removal were injected.

Total amount of discarded plasma, 1,000 c.c.

Blood urea nitrogen, before plasmaphaeresis, 34.5 mg. in 100 c.c.; after plasmaphaeresis, 36.5 mg. in 100 c.c.

Plasma chlorids, before plasmaphaeresis, 5.62 gm. per liter; after plasmaphaeresis, 5.81 gm. per liter.

September 20: The patient feels much better, has slept well and is anxious to carry through the projected treatment. Plasmaphaeresis will be repeated today.

9:55 a. m., 700 c.c. of blood withdrawn into 25 c.c. 8 per cent. sodium citrate.

12:55 p. m., 500 c.c. saline suspension of these red cells injected. On finishing this infusion 650 c.c. of blood were withdrawn through the same needle and treated as in the first instance.

4 p. m., 650 c.c. saline suspension of washed cells from second removal were injected.

Total amount of discarded plasma, 900 c.c.

Blood urea nitrogen, before plasmaphaeresis, 34.7 mg. in 100 c.c.; after plasmaphaeresis, 35.6 mg. in 100 c.c.

Plasma chlorids, before plasmaphaeresis, 5.81 gm. per liter; after plasmaphaeresis, 6.03 gm. per liter.

September 21: Patient much improved and encouraged. He has had no unfavorable reaction of any sort from his two experiences with plasmaphaeresis.

September 24: Blood urea nitrogen, 30.9 mg. in 100 c.c.

Blood: Hemoglobin, 53 per cent.; red blood count, 2,848,000; white blood count, 13,300. Smear: polymorphonuclears, 86 per cent.; lymphocytes, 10 per cent.; large mononuclears, 4 per cent. Anisocytosis and achromia present as formerly.

September 25: The patient has been out of bed in a wheel chair. He now sleeps without orthopnea. Plasmaphaeresis will be repeated.

8:15 a. m., 800 c.c. of blood withdrawn into 25 c.c. 8 per cent. sodium citrate.

11:35 a. m., 575 c.c. saline suspension of these red cells injected. On finishing this infusion 700 c.c. of blood were withdrawn through the same needle and treated as in the first instance.

2:15 p. m., 575 c.c. saline suspension of washed cells from second removal injected.

Total amount of plasma discarded, 1,000 c.c.

Blood urea nitrogen, before plasmaphaeresis, 33.2 mg. in 100 c.c.; after plasmaphaeresis, 33.5 mg. in 100 c.c.

Plasma chlorids, before plasmaphaeresis, 5.26 gm. per liter; after plasmaphaeresis, 5.32 gm. per liter.

Urine: Amber, pale, slightly turbid, acid. Specific gravity, 1.006; large trace of albumin; no sugar. Sediment: occasional epithelial cell, rare red blood corpuscles; occasional granular cast.

September 28: The patient's anemia has not changed since entrance, and transfusion will be done as soon as a donor can be found. He has been up and about the ward for the last four days and has not felt any symptoms except fatigue. Systolic blood pressure remains constantly at 220 mm. Hg.

October 1: Patient received a transfusion of the washed red cells from 600 c.c. of blood from a properly matched donor. These cells were suspended in plasma from the same donor which had been cleared of formed elements by one hour of high speed centrifugalization. He had a very slight chill and temperature reaction following this transfusion.

Blood urea nitrogen, before transfusion, 40.7 mg. in 100 c.c.; after transfusion, 40.7 mg. in 100 c.c.

Plasma chlorids, before transfusion, 5.69 gm. per liter; after transfusion, 6.06 gm. per liter.

October 6: Blood urea nitrogen, 42.8 mg. in 100 c.c. Plasma chlorids, 5.53 gm. per liter.

October 13: The patient has remained constantly in bed since October 7 and has been failing steadily. Plasmaphaeresis again carried out.

9:30 a. m., 500 c.c. of blood withdrawn into 25 c.c. 8 per cent. sodium citrate.

12:30 p. m., 500 c.c. saline suspension of these red cells injected. On finishing this infusion 500 c.c. of blood were withdrawn through the same needle and treated as in the first instance.

3:30 p. m., 500 c.c. saline suspension of red cells from second removal injected; 600 c.c. of blood were withdrawn through the same needle and treated as usual.

6:15 p. m., 600 c.c. saline suspension of red cells from third removal were injected.

Total plasma discarded, 1,000 c.c.

Blood urea nitrogen, before plasmaphaeresis, 92.3 mg. in 100 c.c.; after first red cell return, 92.3 mg. in 10 c.c.; after second red cell return, 95.7 mg. in 100 c.c.

Blood chlorids, before plasmaphaeresis, 4.84 gm. per liter; after first red cell return, 4.66 gm. per liter; after second red cell return, 5.19 gm. per liter.

October 14: Blood urea nitrogen, 109.4 mg. in 100 c.c. Blood chlorids, 5.31 gm. per liter.

October 15: Yesterday and today the patient has been much more drowsy than previously and today he has taken little nourishment. His systolic blood pressure, previously from 200 to 220 mm. Hg, is now 270. There is no edema or ascites.

October 16: Patient has been comatose for the last twenty-four hours. Systolic blood pressure this afternoon, 290. Died at 12:15 a. m., October 17.

DISCUSSION

Plasmaphaeresis, in so far as it was carried in this case, has not arrested the march of uremia in any degree. The encouraging betterment which is noted early in the patient's stay in the hospital is no more than one often sees from rest and proper diet. Whether plasmaphaeresis can be carried to greater extent remains to be seen, but it seems improbable that real good can come from it in chronic cases with impending uremia. The other patients on whom we have used the maneuver have been of similar type and have received no benefit from it or from blood transfusion. It is possible that a case of acute nephritis with suppression of urine might be tided through a critical period of impending uremia by repeated plasma removals, but our series does not contain any such case.

It is of some interest to note that the urea nitrogen of the plasma increases slightly during the process of blood dilution. This finding corroborates that of Turner, Marshall and Lamson,[2] and cannot be explained without more complete studies on nitrogenous metabolism than we at present possess.

CONCLUSIONS

1. A method is described which permits repeated withdrawals of blood and return of washed cells without the use of herudin.

2. Plasmaphaeresis of this type has been carried out eighteen times in eight different cases of chronic nephritis and uremia without definite benefit to the patients.

3. It is suggested that the maneuver can have clinical value in no cases save those of acute suppression of urine where it is a question of tiding over a brief period.

POLIOMYELITIS, CLINICALLY ATYPICAL BECAUSE OF COMPLICATING INFECTION BY A PROTEUS-LIKE BACILLUS *

OSCAR T. SCHULTZ, M.D. (CHICAGO)
Captain, M. C., U. S. Army

AND

ARTHUR M. DANNENBERG, M.D. (PHILADELPHIA)
Captain, M. C., U. S. Army

CAMP JACKSON, COLUMBIA, S. C.

Poliomyelitis, fortunately, has thus far been a disease of rare occurrence in the Army. Epidemic outbreaks probably will not occur, because of the immunity which most adults seem to have. Occasional cases may be expected to develop, especially in those who have just come from civil life, and unless they are recognized early and isolated, other cases may occur among the nonimmunes exposed by contact. A case of poliomyelitis contracted before arrival in camp was recently encountered at Camp Jackson, and the clinical course was complicated and the diagnosis rendered difficult by a secondary infection which was the result of the primary disease.

REPORT OF CASE

History.—The patient, an Italian laborer, was admitted to the Base Hospital, Camp Jackson, June 27, 1918, on his arrival from Chicago, complaining of indefinite pains in the left hip, thigh and knee. The temperature was 102 F. He was married, had no pernicious habits and denied venereal infection. His family history was negative. He gave a history of having received, in April, 1918, a blow in the left parietal region which rendered him unconscious. At the hospital to which he was taken he regained consciousness within twenty-four hours. A diagnosis of fractured skull was made from the roentgen-ray findings. He was later discharged from the hospital in an improved condition. He has since then never felt well, but at no time suffered from symptoms suggestive of a focal brain lesion.

Physical Examination.—Examination on admission determined a poorly nourished man, apparently 30 years of age, having a sickly appearance. The pupils were equal and reacted to light and accommodation. The tongue was slightly coated and tremulous. The pharynx was normal. Pyorrhea alveolaris was present. Slight enlargement of the cervical glands was noted. Examination of the heart and lungs was negative, except that the breath sounds had a harsh bronchovesicular quality over the right upper lobe anteriorly. The abdomen was rather full and prominent. No signs of fluid were present. Tenderness was elicited on deep pressure throughout, more marked in the right

* From the laboratory of the Base Hospital, Camp Jackson, Marshall A. Barber, Captain, S. C., U. S. Army, Chief of Laboratory; and the medical service of the Base Hospital, Camp Jackson, W. W. Herrick, Major, M. C., U. S. Army, Chief of Medical Service.

lower quadrant. The liver was not palpable and the spleen was easily felt 1 inch below the costal margin. The reflexes were found to be as follows: patellar absent, Achilles absent, plantar normal, deep reflexes of upper extremities active and equal; Romberg's sign was slightly present. Weakness of both lower extremities, the left greater than the right, was noted. Slight atrophy about the posterior part of the right hip joint was present. Tactile and pain sensation was found not impaired. The urine at the time of admission was reported normal and the white blood count was as follows: total 3,500; neutrophils 52 per cent.; small mononuclears 46 per cent.; eosinophils 2 per cent. Malaria organisms were not found. The diagnosis at that time was deferred. The general condition remained about the same for four days, the temperature remaining around 100 F., then subsiding to normal.

Clinical Course.—July 1 the abdomen was still markedly distended and increased weakness of both lower extremities was present. Atrophy of muscles of the left leg was noted. Standing unsupported was difficult and marked swaying of the body occurred when the eyes were closed. The blood Wassermann was reported negative and another white blood count on this date was as follows: total 3,000; small mononuclears 59 per cent., large mononuclears 3 per cent., neutrophils 38 per cent.

July 5. The patient appeared rather dull. Besides the findings previously noted, the liver was palpated two fingers' breadth below the costal margin and a doughy sensation was present on palpation of the distended abdomen. The temperature varied between normal and 100 F.

July 10. The patient looked chronically ill and complained of general abdominal pain. The tongue was coated and dry, the heart sounds were rapid, rate 100, of fair muscular quality and tone. No murmurs were present. The lungs presented no other definite signs except relative dulness at the bases posteriorly. The abdomen was moderately distended and tender on palpation all over, slightly more marked over the lower one-half. The sharp edge of the liver was palpated 1 inch below the costal margin. No masses were felt nor were signs of fluid present. The rectal findings were negative. The patient developed retention of urine and catheterization was necessary every eight hours or oftener. A surgical consultation was had and a diagnosis of tuberculous peritonitis was entertained. A white blood count on this date was: total 4,200; small mononuclears 25 per cent., large mononuclears 23 per cent., neutrophils 51 per cent., basophils 1 per cent. Malaria organisms were not found. The urine obtained by catheter was negative. The neurologic opinion was that a cord lesion involving the lower motor neuron was present, because of the rapidly advancing flaccidity and loss of muscle power in the lower extremities, occurring without sensory changes and with abolition of the deep reflexes.

July 12. The patient continued to run a temperature of about 100. He looked quite ill, was hyperesthetic and objected to examination. Voluntary movements of the legs were limited to feeble movement of the toes. The patellar and Achilles tendon reflexes remained absent. The neck was moderately stiff and slight weakness of the right face was noted. A tentative diagnosis of anterior poliomyelitis was made by Major W. W. Herrick and the patient was isolated. A lumbar puncture was performed and 8 c.c. of clear straw-colored spinal fluid was removed. This was reported as follows: Spontaneous coagulation in two hours; cell count 90 per c.mm.; small lymphocytes 99 per cent.; large lymphocytes 1 per cent.; globulin 4 plus. No organisms were found and the culture was negative. Wassermann was negative.

July 14. The temperature continued as before and eyeground examination without the use of a mydriatic showed the disk outline in the left eye indistinct with the veins slightly full and the arteries standing out distinctly. No hemorrhage or exudate was present. The right eye was normal.

July 15. Thorough examination for the detection of hip joint and Pott's disease determined no evidence of these diseases present. Ear examination was also negative. No ova were found on feces examination.

July 16. Retention of urine as before noted continued to be present. Bowel movements were sluggish and enemas had to be given frequently. The appetite was poor. A blood culture which was taken on the 12th was reported on this date to be positive for meningococci, as proved by agglutination with polyvalent antimeningococcus serum. Active antimeningococcus treatment was started after another specimen of blood was taken for culture. The latter was subsequently reported negative. A total of 320 c.c. of antimeningococcus serum was given intravenously and 60 c.c. intraspinally in a period of three days. The spinal fluid while under treatment became quite clouded, was reported to contain many pus cells, but at no time were organisms found. While under treatment the patient's general condition improved markedly. He appeared brighter, developed fair appetite and was less complaining. Flaccidity of both lower extremities and distention of the abdomen remained, however, as before. A return of the power to void voluntarily and control the rectal sphincter was a surprising result of the serum therapy. This continued until July 20 when incontinence of urine and feces occurred.

July 18. The patient complained of pains shooting up and down both lower extremities. The serum treatment was discontinued on this date. The general condition was still improved.

July 19. The deep reflexes of the upper extremities were noted to be prompt and equal. The reflexes of the lower extremities remained absent. Tactile and pain sensation was not impaired. Eyeground examination determined no pathologic condition present. No choroid tubercles were found. The urine report on this date was as follows: Neutral, specific gravity 1.003, albumin trace, sugar negative, microscopic examination negative, heavy amorphous deposit present.

July 20. Roentgen-ray report of examination of head, entire vertebral column and chest was reported as follows: No evidence of fracture of skull or tuberculosis of the vertebral column. No infiltration of lungs present. The patient on this date began to do poorly. The temperature rose to 102 and remained irregular, varying between normal and 104 until death. Incontinence of urine with retention continued. Frequent involuntary bowel movements also occurred. Rectal examination determined the loss of the sphincter reflex. Absolute flaccid paralysis of both lower extremities was present. They were cold in comparison to the upper limbs. The abdominal reflexes were absent. The heart sounds showed nothing definite except impairment of muscular quality. The lungs were clear.

July 23. The white blood count was: total 3,200; small mononuclears 51 per cent.; eosinophils 4 per cent.; neutrophils 39 per cent.; basophils 1 per cent. The patient looked quite ill and presented the general features of a chronic illness. He had frequent desires to void, though the bladder was not full. The urine report was as follows: Cloudy, straw-colored, alkaline, specific gravity 1.004, albumin present, sugar negative, microscopic examination negative except for slight excess of epithelial cells.

July 26. The patient had a chill, and blood examination for malaria at that time was negative.

July 27. A lumbar puncture was performed and 30 c.c. of clear fluid was obtained under slight pressure. It was reported as containing 30 cells per c.mm., globulin 4 plus, no organisms, no tubercle bacilli, and the fluid was anticomplementary for the Wassermann reaction. Urine report of this date: cloudy, straw-colored, alkaline, specific gravity 1.000, albumin trace, sugar negative, microscopic examination negative.

July 29. The patient appeared very weak but did not complain of any pain. Sphincter control of the bladder and rectum was entirely absent. Pos-

teriorly over the bases of both lungs signs of congestion were noted. The patient became weaker and died on Aug. 1, 1918.

To summarize briefly, the case was one which presented (1) a rapidly developing flaccid paralysis of both lower extremities, with loss of control of the bladder and rectal sphincters, and without any sensory changes; (2) a terminal sepsis without characteristic blood changes of a sepsis, and urine which remained apparently normal; and (3) a spinal fluid suggestive from its cell count of poliomyelitis or tuberculous meningitis, but misleading because of the presence of xanthochromia and spontaneous coagulation which were suggestive of an old subdural hemorrhage, or brain or cord tumor.

Necropsy.—From the record of the necropsy, which was held fifteen hours after death, the following notes are taken:

The right pupil is contracted, the left is in middilatation. The legs and thighs are small, apparently because of atrophy of muscles. The abdomen is distended. Peritoneal, pleural and pericardial cavities are normal. The heart, lungs, liver, adrenals, and gastro-intestinal tract show nothing which deserves special mention.

The spleen is enlarged to between four and five times the normal size. The capsule is smooth and bluish red in color, and the organ feels firm. On section the cut surface is dark red in color and shows a number of opaque, yellowish white, round bodies, from 1 to 2 mm. in diameter. These are dry and friable and shell out readily from the surrounding tissue. The malpighian bodies are visible.

Each kidney is enlarged to almost double the normal size. The pelves are moderately distended and the ureters are 1 cm. in diameter. The surface of each kidney shows numerous slightly raised, nodular, opaque, grayish areas from 1 to 3 mm. in diameter, some of which are surrounded by areas of congestion. On section the cortex is thickened and is traversed by numerous, opaque, grayish streaks which end superficially in the areas noted on the surface. The pelves and ureters contain yellow, cloudy fluid.

The inner surface of the calvarium is normal and shows no evidence of previous fracture. The dura is not abnormally adherent to the bone. The inner surface of the dura over the left cerebral hemisphere is covered by a thin, rather firmly adherent layer of dark red material; over the parietal lobe this is yellowish in color. The pia is normal. There is a slightly increased amount of clear fluid in the sulci of the parietal lobes. The dura and pia of the base of the brain and the base of the skull appear normal. On section the brain appears normal.

The veins of the lumbar cord and of the cauda equina are markedly distended. The membranes of the cord are normal. On section the anterior horns of the gray matter of the lumbar cord stand out prominently, are pink in color, and appear to be softer than normal. The rest of the cord is normal on section.

Microscopic Examination.—The following notes are taken from the record of the microscopic examination:

Liver: The tissue contains numerous cellular islands, composed chiefly of lymphocytes. In these areas the liver cells are degenerated or necrotic or have disappeared completely; most of the islands are interlobular, but some are intralobular. There are a few small areas in which the liver cells are necrotic, but no cellular infiltration is present.

Spleen: The tissue is moderately congested. The opaque yellowish areas noted in the gross are composed of structureless, granular, eosin-stained material, which peripherally contains fine chromatin granules. Externally the mass is surrounded by compressed, congested spleen tissue.

Kidney: Scattered about in the cortex are irregularly shaped, poorly outlined cellular areas, composed chiefly of pus cells. In such areas some tubules and glomeruli are completely necrotic, other tubules are filled with pus cells,

and still others have disappeared completely. The tissue of the pelvis is diffusely infiltrated by pus cells.

Dura: The dura of the left cerebral hemisphere is normal, but beneath it is a layer of new formed tissue almost double the thickness of the dura. The fibers of this tissue are dense. The capillaries are wide and thin walled. In the tissue are numerous swollen, rounded and elongated cells which are filled with coarsely granular, dark brown pigment. Many well preserved red corpuscles are free between the fibers of the tissue. Just beneath the dura the tissue contains young fibroblast nuclei and a few lymphocytes.

Brain: The pia is edematous and contains moderate numbers of round and spindle cells which are filled with granular pigment which is not so dark as that present in similar cells in the tissue beneath the dura. The brain tissue is normal.

Cord: In the lumbar cord the ganglion cells of the anterior horns show varying degrees of degeneration. Some contain finely granular brown pigment and have no nuclei. Others contain numerous large, clear vacuoles. Still others are granular and completely broken up. Most of the cells which are better preserved contain large chromatin staining granules of the appearance and distribution of Nissl bodies. Lymphocytes in moderate numbers are present in both gray and white matter. Neurophagocytosis is not marked; an occasional degenerated ganglion cell is surrounded by a few lymphocytes. The capillaries of the anterior horns are moderately distended. In the pia, lymphocytes are fairly numerous, being especially aggregated about the blood vessels. In the cervical cord an occasional degenerated ganglion cell is present in the anterior horns.

Diagnosis.—The anatomic diagnosis summarizes the findings as follows: Subacute poliomyelitis of lumbar cord. Atrophy of muscle of lower extremities. Multiple abscesses of both kidneys. Bilateral pyoureter and pyonephrosis. Organizing subdural hemorrhage of left cerebral hemisphere. Chronic hemorrhagic pachymeningitis of left cerebral hemisphere. Acute splenic tumor. Cloudy swelling and multpile abscesses of liver.

Bacteriology.—Cultures were made from the hemorrhagic dura, the subpial fluid, the kidney abscesses, and the spleen. In all there developed a slender, motile bacillus, the cultural reactions of which were as follows:

Broth: In twenty-four hours, uniformly clouded, with no sediment or pellicle. In forty-eight hours a thin pellicle is formed.

Peptone Water: Growth is the same as in broth; no indol is formed.

Litmus Milk: Not changed in twenty-four hours. In forty-eight hours the medium is acid throughout and is peptonized at the surface. In seventy-two hours the litmus is almost completely decolorized and the upper fourth of the milk is peptonized. In five days, completely decolorized, almost completely peptonized, with a putrefactive odor.

Loeffler's Blood Serum: Partially liquefied in forty-eight hours, the liquefaction becoming complete later.

Dextrose Litmus Agar: Acid and gas in twenty-four hours. Gas increased in forty-eight hours, the butt partly decolorized. No further change later.

Maltose Litmus Agar: In twenty-four hours the butt is acid but no gas is formed. In forty-eight hours the butt is decolorized; no gas has been formed.

Lactose Litmus Agar: No change.

Saccharose Litmus Agar: No change in twenty-four hours. In forty-eight hours the butt is decolorized without gas formation.

Mannite Litmus Agar: No change.

Inulin Litmus Agar: Butt decolorized without gas formation in twenty-four hours; no further change later.

The organism appears to be a member of the proteus group, although the absence of coagulation of milk, of indol formation, and of saccharose fermentation is atypical for the usual strains of *B. proteus.*

DISCUSSION

The complicating infection, which made this case so puzzling clinically, was such a one as might be expected to occur with the spinal cord involvement situated where it was. Given paralysis of the bladder and rectum, with repeated catherization and frequent involuntary defecation, and one rather expects ascending secondary infection of the urinary tract to occur. But the laboratory examinations, which should have cleared up this feature of the case, failed to do so, and there were added other features, some of which are difficult to explain. The involvement of the dura, the result of the injury to the skull three months before admission, was apparently without effect on the clinical complex on admission. The stiffness of the neck and weakness of the face which were noted on July 12 may have been the result of edema and perhaps of diffuse hemorrhage of the thickened dura. The cell count and globulin content of the spinal fluid suggested either poliomyelitis or tuberculous meningitis, but the pigmentation and spontaneous coagulation of the fluid were difficult to correlate with either possibility, and it remained for the necropsy to show that these confusing findings were due to the old subdural hemorrhage. Why the repeated urine examinations failed to detect the pus which must have been present in the urine and the detection of which would have cleared up the nature of the infection which overshadowed the later clinical picture, is difficult to explain except on the ground that the urine was always highly alkaline and that the pus cells may have undergone solution. The continued absence of leukocytosis after the secondary infection developed also helped to confuse the clinical picture, and is difficult to explain except on the basis of a lack of reactive power on the patient's part. The isolation, from blood taken on July 12, of a gram-negative diplococcus which was agglutinated by polyvalent antimeningococcus serum is a most puzzling phenomenon. The spinal fluid at this time was negative culturally, a similar organism could not be obtained at necropsy, and there was absolutely no postmortem evidence of previous localization of the meningococcus in the meninges. Possibly there may have been a blood invasion by the meningococcus, a feature of meningococcus infection the importance of which has been emphasized in the investigations of Major W. W. Herrick, chief of the medical service at this hospital. And this invasion may have yielded to the treatment with specific antiserum before there was any meningeal localization. At this stage the case was clinically one of poliomyelitis.

The temporary improvement in the general condition which followed the intravenous administration of antimeningococcus serum may have been a reaction of the nature of the nonspecific reaction which

follows the introduction of foreign protein in other acute infections. The transient amelioration of symptoms due to cord involvement, after intraspinous serum treatment, may have been the result of stimulation by the serum of only partially degenerated neurons. The case is instructive because of its puzzling features, some of which are difficult or impossible of explanation, and because it emphasizes the importance of not permitting too concentrated consideration of one feature of a case to overshadow other possibilities which may arise.

SUMMARY

A case of poliomyelitis, contracted by a soldier in civil life before his departure for camp, is described.

The earlier clinical course was that of an acute poliomyelitis involving the lumbar cord.

Xanthochromia and spontaneous coagulation of the spinal fluid were present, due to an old subdural hemorrhage which was the result of an injury three months before admission.

The later course was complicated by infection of the urinary tract and by generalized infection, the result of paralysis of the bladder caused by the poliomyelitis.

The organism which caused the secondary infection, while not a typical *B. proteus*, is apparently a member of the proteus group.

THE RELATIVE FREQUENCY IN RECRUITS WITH AND WITHOUT THYROID ENLARGEMENT OF CERTAIN SIGNS AND SYMPTOMS WHICH OCCUR IN NEUROCIRCULATORY ASTHENIA *

THOMAS ADDIS (San Francisco)
Captain, M. C., U. S. Army; Base Hospital
AND
WILLIAM J. KERR (San Francisco)
Captain, M. C., U. S. Army; Chief of Medical Service, Base Hospital
CAMP LEWIS, AMERICAN LAKE, WASH.

SECTION 1.—OBJECT OF THE INVESTIGATION

A considerable number of soldiers under training have been referred to the Cardiovascular Board on account of the presence of a syndrome which included all or most of the following signs and symptoms. The signs were increased pulse rate, tremor of the fingers and cold, moist hands which became cyanosed when dependent. The symptoms were precordial pain with dyspnea and palpitation on moderate exertion, such indications of vasomotor instability as dizziness, flushing and fainting, and a variety of other complaints, all pointing to a state of excessive reaction of the nervous system to psychic or physical strain.

It was the impression of the board that thyroid enlargement was almost constantly found in men with this syndrome. This fact raised the question as to the relationship between the thyroid anomaly and the development of these signs and symptoms. It was clear that they were not cases of exophthalmic goiter if for no other reason than that eye signs were not present. But it is known that in certain cases of endemic goiter a toxic state develops which resembles the condition we have outlined, and it has been shown in a previous report from the board that a high proportion of the men at Camp Lewis suffer from endemic goiter. It seemed possible, therefore, that the unwonted strain of military training had developed in these cases of endemic goiter an unusual number of instances of "toxic goiter." The possibility of holding such an opinion seemed further to be strengthened by the experience at Camp Upton where 75 per cent. of these men

* From the Cardiovascular Board, Camp Lewis, Wash. The statistics for this report were collected while the authors were members of the Cardiovascular Board.

were found to have thyroid enlargement. The condition is there attributed to hyperthyroidism.

But it will be noted that the supposition that an appreciable number of men with this syndrome were instances of "toxic goiter" was only tentatively held because of the impression that the condition was most commonly seen in men with thyroid enlargement. If this impression were a mistaken one there would be little reason for any such hypothesis, for on clinical grounds no distinction could be made between the syndrome in men with thyroid enlargement and those without it, and it seemed that there was a complete parallelism between the signs and symptoms we saw at Camp Lewis and those described under the term "Effort Syndrome" or "Neurocirculatory Asthenia" in England.

The object, therefore, of the survey here reported was to find whether or not the signs and symptoms we have mentioned were more frequent in recruits with thyroid enlargement than in those who had no thyroid enlargement.

SECTION II.—CONDITIONS UNDER WHICH THE FREQUENCY OF SIGNS AND SYMPTOMS IN THE TWO GROUPS WERE COMPARED

SUBSECTION A.—CLASSIFICATION OF RECRUITS INTO GROUPS

1. Classification According to the Absence or Presence of Thyroid Enlargement.—In some cases the existence of thyroid enlargement was not open to question. These men all had a swelling in the neck which was obvious on inspection and which moved on swallowing. These have been classed as "Certainly enlarged thyroids."

In other cases palpation during swallowing failed to reveal even the isthmus of the thyroid gland. These have been classed as "non-thyroids."

In still other cases, not inconsiderable in number, different observers might have disagreed as to whether or not thyroid enlargement were present. The isthmus was palpable, and in thin-necked individuals even visible. We are inclined to the view that in most of these cases the thyroid was enlarged beyond the limits of physiologic variation, but this was a point into which the element of judgment entered, and in a statistical study such as this in which a considerable mass of data was available, it seemed better to classify them separately as "Possibly enlarged thyroids."

2. Classification According to the Origin of the Recruits from Districts where Endemic Goiter is Prevalent or Relatively Uncommon.—So far as we know, there is no necessary correlation between the degree of thyroid enlargement and the occurrence or severity of the condition of toxic goiter. It is conceivable that in districts where

endemic goiter is prevalent a certain proportion of individuals may present more or less marked toxic symptoms, in whom there is no detectable or no obvious thyroid enlargement. Because of this possibility recruits free from any certain thyroid enlargement might not form a reliable normal standard for comparison with those in whom the thyroid enlargement was present, if they came from a district where endemic goiter was common. The normal standard, other things being equal, should be selected from districts free from endemic goiter. This was not possible since goiter is present in all the states from which recruits for Camp Lewis are drawn. But there are very marked differences in the frequency of the condition. Thus, only 8 per cent. of recruits from California had "possibly" or "certainly enlarged thyroids," while the percentage in Washington and Oregon was 39 per cent. and 32 per cent., respectively.

We have accordingly drawn a comparison between the incidence of signs and symptoms in recruits from California and in recruits from Washington and Oregon.

SUBSECTION B.—THE CONDITIONS OF EXAMINATION

A previous attempt to collect these data had shown that it was essential that the examination should be made before the men had received their first injection of typhoid vaccine, since that circumstance was in itself a sufficient cause for the development of some of the signs we were investigating. During the June draft, accordingly, an attempt was made to examine the men in the interval between the examination for communicable diseases and the injection of vaccine. This interval was short and was broken into to a considerable extent by the necessity of organizing the men in companies. As a result, data were obtained on only 314 men. These, however, were seen under satisfactory conditions, between 9 a. m. and 12 noon in their barracks. A squad room was filled with men who were directed to lie on their bunks flat on their backs without moving until they had been seen. No one was examined until they had lain quiet for at least five minutes.

During the July draft the investigation was greatly facilitated by a camp order under which the typhoid vaccination was postponed until the men were mustered in. Even then it was difficult, because of the absence of the men at the examining boards or the fitting of uniforms and the carrying out of preliminary drills, to see many men during the day, and the examination was therefore carried through between 6 p. m. and 9 p. m., after the men had been released from duty. The same plan of examining only after a period of complete rest in the recumbent position was followed.

SECTION III.—RELATIVE FREQUENCY OF CERTAIN ISOLATED SIGNS IN RECRUITS WITH AND WITHOUT THYROID ENLARGEMENT

SUBSECTION A.—INCREASED PULSE RATE

The pulse rate is determined by so many different factors that there can obviously be no generally applicable definition of what constitutes an increase over the normal in pulse rate. All that can be done is to determine the variability of the rate in normal individuals under certain arbitrary fixed conditions. The necessity for establishing some such standard, not only for the classification of cases dealt with in this report, but for more general use, has led to a separate study which will shortly be reported in detail. The basis chosen for this standard was the regulation hopping exercise test, but it was found necessary to modify this by preceding it by a period of rest in the recumbent position and by taking the first pulse count with the subject still lying down.

The average pulse rates under the conditions of this modified exercise test for the three groups of "Certainly enlarged thyroids," "Possibly enlarged thyroids" and "Nonthyroids" defined in Section II are given in Table 1.

TABLE 1.—AVERAGE PULSE RATES OF GROUPS OF RECRUITS CLASSIFIED ACCORDING TO THE PRESENCE OR ABSENCE OF THYROID ENLARGEMENT

Thyroid Enlargement	No. Recruits	Lying	Standing	After Hopping 100 Times	Two Minutes After Hopping 100 Times
Certainly enlarged thyroids..	143	76	92	104	81
Possibly enlarged thyroids...	533	77	93	106	81
Nonthyroids	900	78	95	108	83

The figures in Table 1 show very definitely that thyroid enlargement in a group of individuals is not associated with any increase in the average pulse rate of the group, but it is still possible that in certain cases within the group the thyroid abnormality might have induced an increase in pulse rate, and that this increase might be of a distinctive nature, appearing, for example, to a greater degree in one or other of the different stages of the test. Those cases which showed abnormalities of pulse rate as determined by comparison with the pulse rate standard previously referred to, were therefore separated from the others and individually examined.

The number and percentage of the cases in each group presenting pulse rate abnormalities is given in Table 2.

The average pulse rate in the abnormal cases is given in Table 3.

It is not shown by either Table 1 or Table 2 that there is any characteristic difference in men who have abnormal pulse rates between those with and those without thyroid enlargement. This conclusion is

further supported by the results obtained when the deviations of each abnormal case from the standard are averaged. These are given in round figures in Table 4.

TABLE 2.—Number and Percentage of Recruits with Abnormalities of Pulse Rate

Thyroid Enlargements	No. Recruits	No. Abnormalities	Percentage of Abnormality
Certainly enlarged thyroids	143	58	41
Possibly enlarged thyroids	533	194	36
Nonthyroids	900	372	41

TABLE 3.—Average Pulse Rates of Recruits with Abnormalities of Pulse Rate

Thyroid Enlargement	Lying	Standing	After Hopping 100 Times	Two Minutes After Hopping 100 Times
Certainly enlarged thyroids	80	100	116	89
Possibly enlarged thyroids	83	103	118	90
Nonthyroids	84	104	119	91

TABLE 4.—Average Excess Over the Standard In Cases with Abnormalities of Pulse Rate

Thyroid Enlargement	Lying	Standing	After Hopping 100 Times	Two Minutes After Hopping 100 Times
Certainly enlarged thyroids	7	7	11	5
Possibly enlarged thyroids	8	7	8	5
Nonthyroids	9	7	8	6

No importance can be attached to the deviation of eleven after hopping in the group of certainly enlarged thyroids, since this is the average of only sixteen men, one of whom showed an exceptionally high rate. There is then no indication that thyroid enlargement is associated with any special type of pulse rate.

The comparison between pulse rates of men from California and from Washington and Oregon where endemic goiter is most common, is vitiated by the fact that the average Californian rate was higher than the Washington and Oregon rate. It is because of the large number of Californians in the general nonthyroid group that the rate in the nonthyroid group of Table 1 is somewhat higher than the rates in the thyroid groups. The most accurate comparison is therefore between thyroids and nonthyroids coming from the same states. This is given in Tables 5 and 6.

It is apparent from Tables 5 and 6 that whenever a sufficiently large number of data are available the pulse rates are practically the same in both thyroid and nonthyroid groups. Nor is it possible to

detect any appreciable or constant difference in thyroid as compared with nonthyroid cases with abnormal pulse rates when the comparison is made between men from the same state.

TABLE 5.—AVERAGE PULSE RATES OF RECRUITS FROM WASHINGTON AND OREGON

Thyroid Enlargement	No. Recruits	Lying	Standing	After Hopping 100 Times	Two Minutes After Hopping 100 Times
Certainly enlarged thyroids..	33	77	89	102	80
Possibly enlarged thyroids...	147	77	90	104	79
Nonthyroids	132	76	90	104	79

TABLE 6.—AVERAGE PULSE RATES OF RECRUITS FROM CALIFORNIA

Thyroid Enlargement	No. Recruits	Lying	Standing	After Hopping 100 Times	Two Minutes After Hopping 100 Times
Certainly enlarged thyroids..	22	79	94	113	88
Possibly enlarged thyroids...	188	80	96	108	84
Nonthyroids	497	80	97	109	84

SUBSECTION B.—TREMOR

Tremor was defined as a fine shaking or unsteadiness of the fingers when the arm and hand were extended at right angles to the body. Only those cases were called positive in which the tremor involved all the fingers. Coarse, incoordinate movements of the whole hand or of individual fingers were not classed as tremors.

The number and percentage of tremors found in the three groups of recruits are given in the tables following.

TABLE 7.—NUMBER AND PERCENTAGE OF RECRUITS WITH TREMOR

Thyroid Enlargement	No. of Recruits	No. of Tremors	Percentage of Tremors
Certainly enlarged thyroids.....	143	44	31
Possibly enlarged thyroids.....	532	88	17
Nonthyroids	900	92	10

It would seem from Table 7 that there is a relation between thyroid enlargement and tremor, for tremor is three times more frequent in the group with certainly enlarged thyroids, and nearly two times more frequent in those with possibly enlarged thyroids, than in the nonthyroid group.

This association between thyroid enlargement and tremor is more evident when the comparison is confined to recruits from Washington and Oregon, the two states in which endemic goiter is most prevalent (Table 8).

In California, the state in which endemic goiter is least common, the percentage of tremor among the nonthyroid group is much higher. This may be connected with the fact that the majority of the Cali-

fornians were from cities (San Francisco, Los Angeles, Stockton, etc.), while nearly all the Washington and Oregon men were from the country districts.

TABLE 8.—Number and Percentage of Recruits from Washington and Oregon with Tremors

Thyroid Enlargement	No. of Recruits	No. of Tremors	Percentage of Tremors
Certainly enlarged thyroids	33	5	15
Possibly enlarged thyroids	147	16	11
Nonthyroids	132	3	2

TABLE 9.—Number and Percentage of Troops from California with Tremor

Thyroid Enlargement	No. of Recruits	No. of Tremors	Percentage of Tremors
Certainly enlarged thyroids	22	5	23
Possibly enlarged thyroids	188	19	10
Nonthyroids	497	47	9

The highest percentages of tremor were found in the June draft (Table 10).

TABLE 10.—Number and Percentage of Recruits in the June Draft with Tremor

Thyroid Enlargement	No. of Recruits	No. of Tremors	Percentage of Tremors
Certainly enlarged thyroids	82	31	38
Possibly enlarged thyroids	150	44	29
Nonthyroids	181	33	18

The greater percentage in this group may possibly arise from an unconscious change in the standard adopted by the examiner as to what degree of unsteadiness should be termed "tremor." The fact remains that in every subdivision of the total number of men examined, those with certainly enlarged thyroids showed a relatively higher percentage of tremors than the nonthyroid group. This constant difference is sufficiently large to make it appear that a relation exists between enlargement of the thyroid gland and the occurrence of tremor.

SUBSECTION C.—CYANOSIS OF THE HANDS

The conditions under which examination was conducted were unfavorable for the detection of this sign. The previous experience of the Board in the examination of recruits with thyroid enlargement referred on account of cardiac symptoms had given rise to the impression that cyanosis of the hands was almost constant in such cases. These men were seen while they were standing, and the dependent position of the hands undoubtedly favored the delay in the circulation which is the cause of the cyanosis. But in this survey the men

had been lying for some time in bed with the arms in a horizontal position, and this doubtless is the reason why both in thyroid and nonthyroid cases the frequency of blue hands was so much smaller than that found in the comparison made between these groups in recruits of the May draft as they were in transit between examining boards. It was found that even when present it was not as a rule well marked and so much difficulty was experienced in deciding on borderline cases that it was decided after the June draft to postpone further inquiries into this point until more favorable conditions were obtained. The figures obtained during the June draft are given in Table 11.

TABLE 11.—NUMBER AND PERCENTAGE OF RECRUITS WITH CYANOSIS OF THE HANDS

Thyroid Enlargement	Number Recruits	Number Cyanosed Hands	Percentage Cyanosed Hands
Certainly enlarged thyroids	82	15	18
Possibly enlarged thyroids	150	7	5
Nonthyroids	181	13	7

The numbers examined are too small to allow of any definite conclusion, though the larger percentage found in the group of certainly enlarged thyroids favors the hypothesis of a relationship between cyanosis of the hands and endemic goiter.

SUBSECTION D.—CURVED NAILS

By curved nails is meant an anteroposterior curving. A slight anteroposterior curve is extremely frequent in all individuals. It is commonly most marked in the nail of the index finger and becomes progressively less pronounced in the third, fourth and fifth finger nails. Only those cases were noted as having curved nails in which the fourth nail was very considerably curved. This is a more rigorous definition than was used in the comparison between thyroid and nonthyroid cases in the May draft and the figures are therefore not comparable.

The results obtained in the examination of the June draft are given in Table 12.

TABLE 12.—NUMBER AND PERCENTAGE OF RECRUITS WITH CURVED NAILS

Thyroid Enlargement	Number Recruits	Number Curved Nails	Percentage of Curved Nails
Certainly enlarged thyroids	82	25	31
Possibly enlarged thyroids	150	43	29
Nonthyroids	181	40	22

No further observations on this point were made, as it was felt that no certainty could be attained by purely statistical methods in regard to a matter in which it was so difficult even for the same

observer to maintain a constant standard. As in the case of cyanosis of the hands, a large proportion of the cases were only doubtfully classified.

To any one whose clinical work has been confined almost entirely to the inhabitants of cities, the cyanosis of the hands and curving of the finger nails seen in recruits is at first regarded as a definite and probably significant abnormality. But this impression goes when it is discovered how frequently these signs are found. It is believed, though no figures are available to test this impression, that the cyanosis is most frequent in recruits from the country, whose work has entailed much exposure of the hands to cold with a consequent gradual loss of vasomotor control and a condition of constant dilatation of the capillaries and small vessels so that when their hands are dependent there is a delay in the rate of flow of blood. It may be that the curving of the nails is a secondary result of this circulatory stasis.

The higher percentage of both cyanosis of the hands and curving of the nails in cases with certainly enlarged thyroids, suggests that there may be some association between enlargement of the thyroid gland and loss of vasomotor control in the vessels of the hands.

SUBSECTION E.—MOIST HANDS

By moist hands is meant any perceptible degree of moisture on the palm of the hand.

The significance of this sign of course varies with the condition of the examination. On a close, warm day it may be almost universal. But as the thyroid cases were well distributed throughout the whole group of men examined, the determination of the relative incidence of this sign was not thereby affected, though it is probable that if it had been possible to examine the men during cold dry weather more valuable results would have been obtained. A pronounced clamminess or wetness of the palms of the hands even when the air is cool and dry is certainly very frequent in men presenting the syndrome of neurocirculatory asthenia. The results obtained in the examination of the June and July drafts are given in Table 13.

TABLE 13.—NUMBER AND PERCENTAGE OF RECRUITS WITH MOIST HANDS

Thyroid Enlargement	Number Recruits	Number Moist Hands	Percentage of Moist Hands
Certainly enlarged thyroids	143	99	69
Possibly enlarged thyroids	533	339	64
Nonthyroids	900	553	61

The relatively large number of men examined gives value to the differences noted in Table 13 even though they are not marked. It

is evident, however, from the high percentage of nonthyroid men with moist hands that the relation of thyroid enlargement to this sign, if it exists at all, must be very indirect. A comparison of the percentage of moist hands in recruits from California — a relatively thyroid-free state in which most of the men examined were inhabitants of cities — with the percentage from Washington and Oregon — states where endemic goiter is most common, and in which the men examined were practically all loggers or farmers — suggests that the place of origin or the character of work are factors in the development of this condition (Tables 14 and 15).

TABLE 14.—NUMBER AND PERCENTAGE OF RECRUITS FROM CALIFORNIA WITH MOIST HANDS

Thyroid Enlargement	Number Recruits	Number Moist Hands	Percentage of Moist Hands
Certainly enlarged thyroids	22	16	73
Possibly enlarged thyroids	188	127	68
Nonthyroids	497	300	60

TABLE 15.—NUMBER AND PERCENTAGE OF RECRUITS FROM WASHINGTON AND OREGON WITH MOIST HANDS

Thyroid Enlargement	Number Recruits	Number Moist Hands	Percentage of Moist Hands
Certainly enlarged thyroids	33	19	58
Possibly enlarged thyroids	146	83	57
Nonthyroids	132	67	51

SUBSECTION F.—DERMATOGRAPHIA

A centrifuge tube with a round, blunt, smooth end was drawn in as uniform a manner as possible over the chest. A minute or two later the skin was examined and if a well defined red line were seen the case was noted as presenting dermatographia. Only men of the June draft were examined (Table 16).

TABLE 16.—NUMBER AND PERCENTAGE OF RECRUITS WITH DERMATOGRAPHIA

Thyroid Enlargement	Number Recruits	Number of Cases with Dermatographia	Percentage of Cases with Dermatographia
Certainly enlarged thyroids	82	31	38
Possibly enlarged thyroids	150	59	39
Nonthyroids	181	58	32

Table 16 shows that with this rough and rather inaccurate method there was no marked difference in the incidence of dermatographia in the nonthyroid and thyroid groups.

SECTION IV.—THE RELATIVE FREQUENCY OF CERTAIN SYMPTOMS IN RECRUITS WITH AND WITHOUT THYROID ENLARGEMENT

The symptoms which were considered may be divided into three groups. Dyspnea, palpitation and precordial pain are classed as cardiac symptoms. Dizziness, flushing and fainting are taken as indications of vasomotor instability. Mental irritability, emotionalism, apprehensions, depression, excitability and exhaustion and "shakiness" after exertion or excitement were all grouped under the heading of nervous instability.

It was not possible in the time available to take a careful or exhaustive history. But the system which was followed probably tended to give too low rather than too high an estimate of the frequency of these symptoms. After examining for thyroid enlargement and for tremor and moist hands, the recruit was asked what occupation he followed. The question "You had no difficulty, so far as your health was concerned, in doing that work, had you?" was then put. If he answered "No" and his work were heavy, no further questions were asked, and the case was marked negative as regards symptoms. If the occupation were a light or sedentary one he was further asked whether he got much exercise outside of his work, whether he could do heavy work if necessary, whether he could run well, etc. These initial questions were all directed towards determining whether or not there was dyspnea on moderate exertion without putting the question directly. If it appeared that dyspnea on exertion was present, an endeavor was made to determine its degree and constancy, and its association with other symptoms. In those cases in which there was a clear history of dyspnea on moderate exertion and the recruit had without prompting complained of some symptoms such as pain round the heart, dizziness or "nervousness," it was found necessary to save time by asking directly as to the presence or absence of the other symptoms which were studied, though the precaution was taken of putting questions in the negative form. It was unfortunately not possible to separate the cases with thyroid enlargement into the two groups of certainly and possibly enlarged thyroids since there were only nine cases of large thyroids with symptoms, a number far too small to have any statistical significance.

The percentage of cases with any of these symptoms in recruits with and without thyroid enlargement is given in Table 17.

TABLE 17.—NUMBER AND PERCENTAGE OF RECRUITS WITH SYMPTOMS

Thyroid Enlargement	Number Recruits	Number with Symptoms	Percentage with Symptoms
All enlarged thyroids	444	40	9
Nonthyroids	719	63	9

In Table 18 is the percentage frequency of the cardiac symptoms of dyspnea, palpitation and precordial pain. Table 19 shows the relative frequency of vasomotor instability and Table 20 the frequency of the symptoms grouped under the term nervous instability.

TABLE 18.—PERCENTAGE OF RECRUITS WITH CARDIAC SYMPTOMS

Thyroid Enlargement	Dyspnea	Palpitation	Precordial Pain
All enlarged thyroids	8	8	6
Nonthyroids	8	8	6

TABLE 19.—PERCENTAGE OF RECRUITS WITH SYMPTOMS OF VASOMOTOR INSTABILITY

Thyroid enlargement	Dizziness	Flushing	Fainting
All enlarged thyroids	7	4	3
Nonthyroids	7	6	3

TABLE 20.—PERCENTAGE OF RECRUITS WITH SYMPTOMS OF NERVOUS INSTABILITY

Thyroid Enlargement	"Nervousness"
All enlarged thyroids	5
Nonthyroids	7

The tables show quite conclusively that these cardiac, vasomotor and nervous symptoms are no more frequent in recruits with thyroid enlargement. Similar comparisons were made on recruits coming from the same states and though the numbers examined were too small to make these data worth recording, they support the conclusion which must be drawn from the consideration of the combined figures.

SECTION V.—RELATIVE FREQUENCY IN RECRUITS WITH AND WITHOUT THYROID ENLARGEMENT OF A SYNDROME RESEMBLING THAT OF TOXIC GOITER OR OF NEUROCIRCULATORY ASTHENIA

In the preceding sections it has been shown that tremor and moisture of the hands are somewhat more common in recruits with thyroid enlargement than in those who are free from it. On the other hand, all the symptoms, and the most important single sign — increase of pulse rate — were equally distributed between the two groups.

But no consideration of isolated signs and symptoms can be so decisive as the direct determination of the incidence of the syndrome itself. For each one of these is seen in many other conditions besides toxic goiter or neurocirculatory asthenia. It is only in association with one another that they become of diagnostic value. It is therefore conceivable that even though the distribution of these signs and symptoms, each considered separately, were uniform in thyroid and nonthyroid cases, it might yet be found that the characteristic association

of signs and symptoms was seen only or chiefly in the group with thyroid enlargement. So far as the object of this survey is concerned, we must attach the more decisive importance to the determination of the percentage of cases in each group which may be regarded as presenting this particular association.

It would be erroneous to assign a uniform numerical value to each sign and symptom and to treat the data in a purely mathematical manner, even if the number of our observations were sufficiently large to allow of the determination of coefficients of correlation. For no rigid definition of what is meant by "association of signs and symptoms" can be given. Though there is something clearcut and definite in the clinical picture presented by toxic goiter or by neurocirculatory asthenia, regarded as a whole, yet in detail there is a confusion of many symptoms differing in kind and in degree, and there is no distinctive sign always present, if the thyroid enlargement and the increased pulse rate of toxic goiter be excepted. It seemed, therefore, more correct to adopt a system of selection which, though necessarily arbitrary, conforms with the general method of clinical diagnosis in placing more weight on certain sections of the evidence and less in others. Now one of the outstanding clinical features in both conditions, though this is more especially true of neurocirculatory asthenia, is the multiplicity of subjective complaints and the paucity or even absence of objective evidence. So it comes about that it is mainly on symptoms and not on signs that diagnosis rests. And the special characteristic of the symptoms is the wide field they cover. It is not only the cardiac or the vasomotor or the nervous system which is at fault, but all three together.

In accordance with these clinical facts the following system of selection was adopted. Association of symptoms alone was first considered. Those cases which presented three or more symptoms, one at least of which had to be indicative of cardiac, one of vasomotor, and one of nervous instability, were enumerated in both thyroid and nonthyroid groups and their relative incidence determined. Subsequently the percentage frequency of the signs in these selected cases was compared with their frequency in thyroid and nonthyroid cases in general, in order to determine the degree to which this symptom-complex was associated with increased pulse rate, tremor, etc.

The number and percentage of recruits with the foregoing association of symptoms are given in Table 21.

So far as the data in Table 21 go there appears to be as high a proportion of men in the nonthyroid as in the thyroid group who may be regarded as presenting the symptom-complex we have defined. But the numbers are so small that during the last two days of the July draft

an endeavor was made to supplement them by examinations carried out in the building where the examining boards are stationed. Two thousand three hundred and sixty-seven men were questioned as to the presence of symptoms. The men went by in single file and each was asked whether his "wind was good." If any complaint were made the recruit was detained for further questioning. If it appeared that there was true dyspnea on moderate exertion not due to such causes as asthma, bronchitis or organic heart disease, a detailed history as to the existence of the train of symptoms we have enumerated was taken. The speed at which the work had to be done probably led to a number of cases being missed, for the proportion of men with the syndrome was found to be only about half as great as in the barracks examination. It was uniform for both thyroid and nonthyroid cases, however, so that it is justifiable to combine both sets of observations. This has been done in Table 22.

TABLE 21.—NUMBER AND PERCENTAGE OF RECRUITS WITH SYMPTOMS OF CARDIAC, VASOMOTOR AND NERVOUS INSTABILITY

Thyroid Enlargement	Number Recruits	Number with Symptoms	Percentage with Symptoms
Enlarged thyroids	444	23	5.2
Nonthyroids	719	44	5.8

TABLE 22.—NUMBER AND PERCENTAGE OF RECRUITS WITH SYMPTOMS OF CARDIAC, VASOMOTOR AND NERVOUS INSTABILITY

Thyroid Enlargement	Number Recruits	Number with Symptoms	Percentage with Symptoms
Enlarged thyroids	1,346	51	3.8
Nonthyroids	2,184	86	3.9

The even distribution of the symptom-complex between thyroid and nonthyroid cases in this larger series seems to be a conclusive argument against the view that thyroid enlargement is concerned in the causation of the condition. Yet it is still possible that it may modify it, so that, for instance, certain symptoms may be found to occur with greater frequency when thyroid enlargement is present. Accordingly, the percentage frequency of the symptoms in cases with the syndrome is compared in Table 23.

TABLE 23.—PERCENTAGE OF SYMPTOMS IN RECRUITS WITH SYMPTOMS OF CARDIAC, VASOMOTOR AND NERVOUS INSTABILITY

	Cardiac			Vasomotor			Nervous
	Dyspnea, Per Cent.	Palpitation, Per Cent.	Precordial Pain, Per Cent.	Dizziness, Per Cent.	Flushing, Per Cent.	Fainting, Per Cent.	Nervousness, Per Cent.
Enlarged thyroids	100	96	82	96	65	45	100
Nonthyroids	100	96	86	96	84	46	100

Table 23 shows that the condition remains the same as regards symptoms irrespective of the presence or absence of thyroid enlargement. It is true that the percentage of complaints of flushing is higher in nonthyroid cases, but no great significance can be attached to such a difference because of the relatively small number of cases examined.

The data obtained in the examining building on the incidence of tremors and moist hands in cases with the symptom-complex are combined with the observations made in the barracks in Table 24.

TABLE 24.—Percentage of Tremors and Moist Hands in Recruits with Symptoms of Cardiac, Vasomotor and Nervous Instability

Thyroid Enlargement	Percentage of Tremors	Percentage of Moist Hands
Enlarged thyroids	49	90
Nonthyroids	30	73

Table 24 shows that these signs are especially associated with the symptom-complex. For the percentage of tremors was 19 per cent. in all thyroid cases, and 10 per cent. in all nonthyroids, as compared with 49 per cent. and 39 per cent. in the similar groups with the symptom-complex. These cases may therefore be regarded as presenting that particular combination of symptoms and signs of which the syndrome under discussion is composed.

It is noteworthy that the difference between the percentages of tremor in all thyroid and nonthyroid cases is relatively about the same as the corresponding difference in the percentages of those with symptoms. It would therefore appear that the difference in the group with symptoms is simply a reflection of a general difference between thyroid and nonthyroid cases, a difference which is quantitatively but not proportionately changed with the coincidence of the syndrome.

Unfortunately, it was not possible during the examinations in the examining building to take pulse counts in a manner uniform with the method adopted in the barracks, and the number of observations on this sign is therefore too small to be decisive.

Of the 23 thyroid cases with symptoms, 13, or 57 per cent., had some abnormality of the pulse rate as judged by our standard; while 19, or 45 per cent., of the 42 nonthyroid cases had increased pulse rates. This difference may well be accidental, for Table 25 shows that there is no appreciable difference between the average pulse rates either of all the cases or of those only which were abnormal.

In general, it would seem justifiable to conclude from the tables in this section that the incidence of the syndrome is relatively the same in thyroid and nonthyroid cases, that the distribution of symptoms is the same, and that such differences as are found in the percentage of

tremors and moist hands are not peculiar to recruits with the syndrome, but are also found in a comparison of all thyroid and nonthyroid cases.

TABLE 25.—Average Pulse Rates of Thyroid and Nonthyroid Cases with Symptoms of Cardiac, Vasomotor and Nervous Instability

	All Cases with Syndrome				Cases with Abnormal Pulse Rate			
Thyroid Enlargement	Lying	Standing	Hopping	After	Lying	Standing	Hopping	After
Enlarged thyroids	83	101	118	90	85	105	127	93
Nonthyroids	85	103	119	90	90	108	127	95

SECTION VI.—SUMMARY

The immediate object of the survey was to find whether those signs and symptoms which occur in neurocirculatory asthenia, and which may also be present in toxic goiter, were more commonly seen in recruits with thyroid enlargement than in those who had no thyroid enlargement. The principle findings in regard to those points are summarized in Table 26.

TABLE 26.—Signs and Symptoms With and Without Thyroid Enlargement

Signs or Symptoms	Percentage With Thyroid Enlargement	Percentage Without Thyroid Enlargement
Increased pulse rate	37	41
Tremor	19	10
Cyanosis of hands	9	7
Curved nails	29	22
Moist hands	65	61
Dermatographia	39	32
Dyspnea on exertion	8	8
Palpitation	8	8
Precordial pain	6	6
Dizziness	7	7
Flushing	4	6
Fainting	3	3
Nervousness	5	7
Symptom-complex	4	4

There is no significant difference in the incidence of cases with increased pulse rate; but tremors, and to a lesser extent, moist hands, are somewhat more frequent in the thyroid than in the nonthyroid group. The number of men examined for cyanosis of the hands, curved nails and dermatographia is not sufficiently large to warrant any definite conclusion.

So far as symptoms are concerned it is apparent that there is no appreciable distinction between the two groups.

What has been termed the "symptom-complex," that is, an association in the same individual of symptoms of cardiac, vasomotor and nervous instability, is as often seen in nonthyroid as in thyroid cases.

Though all the details included in the immediate objective have not been adequately dealt with, yet there appears to be sufficient ground to serve as a basis for an opinion in regard to the larger question to answer which these data were gathered. It was to obtain evidence for or against the supposition that in cases with thyroid enlargement the thyroid anomaly was a direct or predisposing cause of these signs and symptoms that their frequency was compared in groups of recruits classified according to the presence of absence of thyroid enlargement.

The greater proportion of tremors in the thyroid cases must first be considered. In the case at least of tremors, reference to the detailed tables in Section III will show that this finding is so marked and so constant that it cannot be dismissed as accidental. Is it then justifiable to conclude that it is necessarily caused by the thyroid abnormality? Obviously not, for there are many other causes of tremor besides hyperthyroidism. The high percentage in recruits free from any suspicion of thyroid enlargement, and coming from California, the state where endemic goiter is least common, is sufficient to demonstrate this. It is at best an hypothesis whose acceptance or rejection will depend on the presence or absence of other indications of the state of hyperthyroidism found in toxic goiter. Now increased pulse rate is the distinctive clinical sign in toxic goiter. It is distinctive because it is the result of the underlying increase in rate of metabolism which is the fundamental difference between this condition and many others which superficially resemble it. For this reason the uniformity which was found in the pulse rate in thyroid and nonthyroid cases is of greater importance than the difference in the incidence of tremors, and makes it improbable that the enlarged thyroid gland in these cases was responsible for the greater incidence of tremors, or, indeed, was the cause of any other constitutional signs or symptoms.

But, as was pointed out in Section V, much more conclusive evidence is to be derived from a consideration of the relative frequency of cases with the symptom-complex than can be obtained from the incidence of isolated signs and symptoms. It has been shown that signs were considerably more frequent in the group of recruits with the symptom-complex than in all cases combined. Many of these men presented in a fully developed form the syndrome described in the introduction to this report; in all of them it was at least potentially present. If thyroid enlargement were a cause of this syndrome, we should certainly expect to find a large proportion of men with the symptom-complex in the thyroid group. But the distribution is the same in the thyroid and nonthyroid cases. On this ground it may be concluded that the development of toxic goiter is not the cause of the syndrome even in men with enlarged thyroids. The enlargement of the thyroid gland in such cases is incidental and not causative.

Since the thyroid has no demonstrable etiologic relationship to this syndrome, there is no reason why, provisionally at least, a diagnosis of neurocirculatory asthenia should not be made in all these cases. Nor is there any reason why cases of thyroid enlargement with the syndrome should be considered as in any essential respect different from those without thyroid enlargement, nor any reason why the treatment and the disposition of these cases should necessarily be altered by the thyroid enlargement as such.

The starting point of this work was the impression that thyroid enlargement was almost constantly present in cases with the syndrome. This impression has not been confirmed. To some extent it was probably erroneous. But it should be remembered that it was obtained from examinations, not of recruits, but of soldiers who had been for some months at Camp Lewis. In the course of a correspondence on this subject, Dr. H. S. Plummer said that he was convinced that thyroid enlargement was more prone to occur in cases of neurocirculatory asthenia than in normal individuals. Since Camp Lewis is situated in a district where endemic goiter is extremely prevalent, it may be that some of these soldiers with neurocirculatory asthenia developed thyroid enlargement only during their stay in camp. Undoubted instances of the new development of goiter in normal individuals stationed at Camp Lewis have been noted.

STUDIES OF THE CHEMISTRY OF PERNICIOUS ANEMIA *

MAX KAHN, M.D., PH.D., AND JOSEPH BARSKY, M.D.
NEW YORK

Squier[1] has written an interesting and comprehensive review of the literature on the chemistry of pernicious anemia. Though a number of researches have been made on the pathochemistry of this very fatal disease, its causative factor is still unknown. Whether it is essentially a disease of the gastro-intestinal tract, with atrophy of the gastric mucosa and the absorption either of enterogenous poisons or protein split products, or whether it is a disturbance due to the hemolytic action of toxins elaborated in disease processes, or whether the disease is caused by hypersplenism, are the three main hypotheses in this mooted question.

The evidence brought forward by those authors who favor one or the other of these theories is not conclusive. A large number of chemical substances, such as oleic acid, saponins, phenylhydrazin, b-amino-azolyl-ethyl benzaldehyd, p-oxyphenylethylamin, etc., are violent hemolytic agents, and experimentally such poisons will produce anemia and hemoglobinuria, etc., but no definite proofs have been advanced that such substances are actually present in the idiopathic anemias. Iwao has been able to isolate p-oxyphenylethylamin from autolyzing pancreas, putrefying horse flesh and Swiss cheese. It is quite evident that this base may normally arise in the intestine from putrefying food, and it has been demonstrated by Berthelot and Bertrand[2] that there is an organism in the intestine—*B. aminophilus*—which can produce p-oxyphenylethylamin from tyrosin. Barger and Dale[3] have isolated another hemolytic amin, b-imino-azolylethylamin, from the mucosa of the small intestine of the ox. Grawitz[4] assumed that intestinal stasis was a great cause in the production of pernicious anemia, due to the absorption of poisons from the decay of food in the alimentary canal. Berger and Tsuchiya[5] found that extracts obtained from the mucosa of the intestines of anemic animals have a much stronger hemolytic power than extracts from the mucosa of normal animals. This has, however, been controverted by Ewald and Friedberger.

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* From the Department of Laboratories, Beth Israel Hospital.

1. For complete bibliography see Squier, T. L.: J. Lab. & Clin. Med. **2**: No. 8, 1917.
2. Berthelot and Bertrand: Compt. rend. Acad. Sc. **154**:1463, 1912.
3. Barger and Dale: J. Physiol. **61**:499, 1911.
4. Grawitz: Berl. klin. Wchnschr., 1898, pp. 704, 730.
5. Berger and Tsuchiya: Arch. f. klin. Med. **94**:252, 1908.

McPhedran found that the hemolytic power of organ extracts of cases of pernicious anemia fell entirely within normal limits, and gave no evidence of the presence of hemolytic toxins supposed to be the causative factor of the disease. That the lipin metabolism may be disturbed in the idiopathic anemias has been surmised by a number of authors. Kinnicutt reports two cases of pernicious anemia, which at necropsy showed lipoidosis of the adrenal cortex. This was indirectly and directly confirmed by the investigations of Hueck[6] and of Landau.

It has been thought by Moffitt that hypersplenism as a cause of this disease may be brought about by toxins which reach the spleen through the main splenic arterial blood supply. "Erythrolysis does not take place in the spleen, but in some way the erythrocytes are sensitized and prepared for later destruction in the liver, marrow or lymph glands."

Examination of the Blood. — The bloods of three patients suffering from pernicious anemia were examined for certain constituents, and the results recorded (Table 1). We shall discuss several of the items individually. We have confirmed the findings of Ruttan and Adami (1896) that the specific gravity of the blood serum is lowered, that the total serum protein is much reduced—about 30 to 40 per cent.—and that the ash was somewhat increased. The calcium in the blood was above normal. The glucose and cholesterol of the blood were normal as well as the total fat content. In this regard we cannot corroborate King's results who found a high blood fat content and a reduced cholesterol content in cases of pernicious anemia.

Study of the Functional Capacity of the Gastro-Intestinal Tract.— The method suggested by Rehfuss for the fractional analysis of the gastric contents was applied, the residuum removed and examined, with the results marked in Table 2. The patients had fasted sixteen hours, from 6 p. m. to 10 a. m.

The residua seem to show a gastric stasis. According to Rehfuss, Bergeim and Hawk the volume of the normal average residuum is about 50 c.c., and shows a total acidity of about 30 and a free acidity of 18.5. In these cases the total acidity averaged 51, whereas the free acid was 0. It may be stated that no pepsin hydrochloric acid was found in the cases of pernicious anemia, while tryptic digestion was present showing a regurgitation of the duodenal contents. The removal of the samples of gastric contents after an Ewald test meal yielded the results in Table 3.

6. Hueck: See Squier, Footnote 1.

TABLE 1.—Blood Analysis in Cases of Pernicious Anemia

	Case 1	Case 2	Case 3
Specific gravity of serum	1.0272	1.0263	1.0259
Water content of blood, parts per thousand	856.12	843.27	839.75
Dry matter of blood, parts per thousand	143.88	156.73	160.25
Proteins of serum, parts per thousand	5.915	5.217	5.337
Fat, parts per thousand	5.05	4.2	4.4
Cholesterol, mg. per 100 c.c. blood	135.0	164.0	155.0
Nonprotein nitrogen, mg. per 100 c.c. blood	38.5	29.3	30.2
Urea nitrogen, mg. per 100 c.c. blood	20.1	18.4	19.7
Creatinin, mg. per 100 c.c. blood	2.8	4.8	5.4
Glucose, mg. per 100 c.c. blood	0.11	0.08	0.09
Uric acid, mg. per 100 c.c. blood	2.2	1.7	3.1
Carbon dioxid, parts per thousand	40.0	45.0 *	38.0
Ash, parts per thousand	0.77	0.82	0.85
Calcium oxid, parts per thousand	0.06	0.087	0.092
Hydrogen-ion concentration* (Pn)	7.2	7.3	7.25

* Oxalated blood.

TABLE 2.—Analysis of Gastric Residium

Case	Volume, C.c.	Total Acid	Free Acid	Pepsin HCl	Trypsin	Bile
1	75	48	0	—	+	Present
2	58	65	0	—	+	—
3	64	40	0	?	+	—

The gastric curves produced by these cases of pernicious anemia strikingly resemble the curves of carcinoma of the stomach. The free acid is absent and the total acid varies between 15 and 45. No blood was present; a trace of lactic acid was found in Case 2; pepsin was absent. The gastric mucosa appears to be altogether nonfunctionating, due to the complete atrophy that is doubtless present of the mucous membrane. The patients (Cases 1 and 2) were complaining of gastric distress, belching and anorexia, and they stated that they felt better when a solution of hydrochloric acid and pepsin was administered to them.

A roentgenogram of the gastro-intestinal tract did not reveal any abnormalities.

The Gastro-albumorrhea test, as devised by Salomon and as modified by Wolff and Junghans, was applied in Cases 2 and 3. The total nitrogen in 100 c.c. of gastric contents was 4.1 mg. (Case 2) and

3.7 mg. (Case 3). No albumin was present as determined by the phosphotungstic acid precipitation method. It is to be assumed that there is no discharge of protein from the gastric mucosa, although there no doubt is a chronic inflammatory process, in quantities sufficient to be tested.

The intestinal digestion was then investigated by the method of Schmidt and Strassburger. The patients were put on the test diet for three days, and the feces, the daily amounts being marked off by carmin, were collected and examined.

TABLE 3.—Fractional Analysis of Gastric Contents

Time, Minutes	Case 1		Case 2		Case 3	
	Free Acid	Total Acid	Free Acid	Total Acid	Free Acid	Total Acid
15	0	36	0	22	0	14
30	0	28	0	24	0	20
45	0	40	0	35	0	25
60	0	26	0	40	0	30
75	0	30	0	45	0	32
90	0	52	0	45	0	32
105	0	56	0	50	0	36
120	0	32	0	52	0	38
135	0	36	0	50	0	40
150	0	36	0	48	0	44
Blood	—		—		—	
Lactic acid	—		Trace		—	
Bile	—		—		—	
Mucus	Normal		Much		Moderate	
Food	Poorly digested		Poorly digested		Poorly digested	
Pepsin	—		—		?	

In a series of six healthy individuals placed on this test diet for three days, Schmidt found the average weight of the dry feces to be 54.3 gm. The maximum was 62 gm. and the minimum was 45 gm. In a case of pancreatic disease, Pratt found the weight of the feces to be above 400 gm. The average weight of the dry feces in five cases of "fermentative dyspepsia," Schmidt found to be 127.4 gm.; the average weight in "gastrogenous diarrhea" with achylia was 98.9 gm.

The analyses of the feces in three cases of pernicious anemia are given in Table 4.

TABLE 4.—Analysis of Feces after Schmidt-Strassburger Test Diet

Case	Day	Weight Dry Feces, Gm.	Total Nitrogen		Fat	
			Gm.	Per Cent. of Intake	Gm.	Per Cent. of Intake
1	1	157.5	2.343	14.2	4.51	4.3
	2	162.4	2.544	15.4	6.05	5.5
	3	175.5	1.897	11.5	5.72	5.2
	Aver.	165.1	2.261	13.7	5.42	5.0
2	1	98.3	1.669	9.7	6.71	6.1
	2	75.5	1.926	11.2	4.29	3.9
	3	108.2	1.857	10.8	5.28	4.8
	Aver.	94.0	1.817	10.5	5.42	4.9
3	1	117.0	1.144	7.2	4.62	4.2
	2	110.5	1.083	6.5	3.96	3.6
	3	86.5	1.319	8.3	4.84	4.4
	Aver.	104.6	1.132	7.3	4.47	4.06

In our series of cases of pernicious anemia the bulk of feces was very much increased, from 75.5 gm. to 175.5 gm. It will be seen that the average daily excretion of dry feces in the three cases was 165.1, 114.0 and 104.6 gm., respectively, an increase of from 100 to 200 per cent. as compared with the normal figures of Schmidt. The nitrogen elimination was also much increased in the first and second cases, being an evidence of some deficiency of protein absorption. The average excretion of nitrogen in the feces in the three cases was 13.7, 10.5 and 7.3 per cent., respectively. The fecal fat does not seem to vary much from the normal, the average figures for the three cases being 5.0, 4.9 and 4.06 per cent., respectively.

Perhaps as index of *intestinal putrefaction* attention may be paid to the urinary sulphur partition. It is known that in cases of intestinal stasis, etc., where the flora of the intestines are abnormal, there is an increased production of aryl compounds which are conjugated in the liver with sulphuric and glycuronic acids, and are excreted in the urine. Normally, the ethereal sulphates form approximately 10 per cent. of the total urinary sulphur. We found, as shown in Table 5, that in the first two cases there was a marked increase in the percentage of ethereal sulphates eliminated, whereas in Case 3, the increase is only slight. To our mind this is a distinct evidence of some putrefactive change going on in the body. We are aware of the theory that a certain portion of the ethereal sulphates is of endogenous origin. Our experience has, however, led us to the conclusion that when marked quantities of ethereal sulphates are

excreted in the urine, presumptive evidence is present of intestinal putrefaction.

It will be noticed that the neutral sulphur fraction is also abnormally high, especially in Case 3, an evidence of body suboxidation.

TABLE 5.—URINARY SULPHUR PARTITION *

Case	Day	Volume Urine, C.c.	Total S, Gm.	Inorganic SO_4		Ethereal SO_4		Neutral S	
				Gm.	Per Cent.	Gm.	Per Cent.	Gm.	Per Cent.
1	A†	1,470	1.892	1.1162	59	0.4351	23	0.3407	18
	B†	1,215	1.536	0.8448	55	0.3968	26	0.2919	19
2	A	1,625	1.074	0.5155	48	0.3014	29	0.2571	23
	B	1,780	1.139	0.6150	54	0.3075	27	0.2165	19
3	A	950	0.935	0.5797	62	0.1496	16	0.2057	22
	B	1,075	1.208	0.7006	58	0.1691	14	0.3383	28

* The total sulphur was determined by Benedict's method, and the inorganic and ethereal sulphates by the method of Folin.
† The days were not successive.

Function of the Pancreas.—An Einhorn duodenal tube was passed on each of the patients and allowed to remain in the patient over night. In the morning the *duodenal contents* were removed and analyzed. We were successful in obtaining bile stained duodenal contents in each instance.

It was found that the pancreatic enzymes were present in abundance. Protease, amylase, and lipase were found in normal quantities. No blood was found in any of the specimens. The methods used were, that of Gross for trypsin, that of Wohlgemuth for amylopsin, and the milk-litmus test for lipase.

In this connection it may be stated that the result of the Schmidt-Strassburger test may be taken as evidence of the nonexistence of any pancreatic deficiency.

The *stools* obtained after the Schmidt-Strassburger diet were examined for the pancreatic enzymes and for blood, besides the usual examination for parasites, food particles, etc.

The pancreatic enzymes were present in abundance—trypsin, amylopsin and steapsin. No blood was found. No ova or parasites were found. Many meat fibers were, however, noticed. No free fat was observed.

The mucus in the stools varied. In one case the mucus was in abundance (Case 1); the other cases were normal.

The *blood* and the *urine* were examined for *amylase,* as suggested by Wohlgemuth, and by Wohlgemuth and Noguchi, as a test for pancreatic insufficiency. We can only report negative results.

The pancreas seems to be functionating entirely normally, as evidenced by the tests enumerated.

Study of the Liver Function.—Bauer's galactose test and *Strauss' levulose test* were applied in two of the cases, with results that were negative in both instances.

Bauer's galactose test is performed as follows:

> Thirty gm. of galactose are administered to the patient in the morning, and the urine collected for the next five or six hours. The presence or absence of galactose in the urine is determined by the Fehling test. The levulose test is applied as follows: One hundred gm. of levulose are administered in the morning, and the urine voided during the following five or six hours, tested by the Fehling and Seliwanoff tests for the presence or absence of levulose.

Sulpho-Conjugation Test.[7]—It was demonstrated by Baumann and others that the toxic carbocyclic radicals split off from the proteins by the growth of intestinal bacteria are conjugated in the liver with sulphuric acid and thus detoxicated. It is also known that a portion of these aryl compounds are detoxicated by conjugation with glycuronic acid.

It is obvious that in testing for the functional capacity of the liver it is essential not only to test the glycogenic, ureogenic, biligenic, etc., functions, but also to examine the detoxicating power of the hepatic tissue in order to ascertain the complete working power of the gland. This is done in the following manner:

> The patient receives a dose of castor oil to evacuate his bowels. He is then kept on a known diet for two days, during which time the urine is collected, preserved and analyzed for total sulphur and ethereal sulphates. On the third day the patient receives a capsule containing 0.5 gm. thymol. The urine is collected for the next two days, preserved, and again analyzed for total sulphur and for ethereal sulphates.

If all the thymol were absorbed, and if all the thymol were conjugated with sulphuric acid and none with glycuronic acid, the 0.5 gm. thymol would be excreted as 0.766 gm. of thymol sulphuric acid. This would cause a marked increase in the percentage of ethereal sulphates in the urine. If the detoxicating power of the liver were below par, the thymol would not be conjugated, and the percentage of ethereal sulphates would be only slightly different from what it had been on the first two days—before the thymol administration.

We have found that this detoxicating function of the liver usually runs parallel with the other functional derangements of this organ.

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7. Kahn, M.: Am. J. M. Sc. **155**:668, 1918.

In some cases, however, the conjugating power of the gland is markedly reduced, whereas the other functions do not show any disturbances as determined by the methods at our disposal. In still other cases the sulpho-conjugation is entirely normal (as determined by the test here described), while the other tests show a reduction of hepatic functional capacity.

It will be seen from the results charted in Table 6 that there seems to be a deficiency in the detoxication function of the liver. While it is seen from the sulphur partition (Table 5) that the liver conjugates an increased amount of aromatic radicals as evidenced by the ethereal sulphate output, still the administration of an additional aryl compound, thymol, does not seem to call forth the proper detoxicating influence of the liver in sufficient force.

TABLE 6.—SULPHO-CONJUGATION TEST OF HEPATIC FUNCTION

Case	Total Sulphur, Gm.		Ethereal Sulphate Sulphur, Gm.		Ethereal Sulphate Sulphur, per Cent. of Total Sulphur	
	Before*	After	Before	After	Before	After
X†	2.0375	2.1295	0.2893	0.5646	14.2	26.8
1	1.536	1.6305	0.3993	0.5055	26.0	31.2
2	1.139	1.0974	0.3075	0.3237	27.0	29.5
3	1.208	1.1522	0.1691	0.2154	14.0	18.7

* Before the administration of thymol, and after.
† This was a normal individual.

Study of the Bile Pigments.—As Squier[1] says, where active erythrocyte destruction is taking place, it is natural to expect that the limit must be approached above which the liver can no longer dispose of the liberated pigment. That such actually is the case has been shown by Sellards and Minot. They found that, compared with normal, less hemoglobin is required to produce hemoglobinuria in patients suffering from pernicious anemia. The amount of hemoglobin necessary to induce hemoglobinuria bears no relation to the red cell count, but is in direct proportion to the amount of blood destruction taking place. This last, providing complicating factors are absent, is directly proportional to the excretion of urobilin in the intestine. Eppinger found that the normal urobilinogen excretion was 0.12 to 0.15 gm. per day. Schneider[7] estimated the amount of hemolysis by examination of the duodenal contents and found in pernicious anemia, excessive excretion of bile pigments, or pleochromie, and urobilinocholia. Pleochromie is an expression of immediate hemolysis, and which, in pernicious anemia, whether in crisis or remission, is a constant finding. Urobilinocholia indicates a heaping

up of pigment in the portal system and varies directly as the portal system is surcharged or becomes relatively empty of the excess of pigment. Schneider[8] points out that in secondary anemia no pleochromie or urobilinocholia is present (Squier[1]).

We followed Schneider's technic in detail. The duodenal contents were collected and examined for the pigments as follows:

To determine bilirubin: To 10 c.c. of duodenal contents are added 10 c.c. of an alkaline solution of calcium chlorid. After vigorous shaking this is filtered. The precipitate is dissolved under gentle heat in 10 c.c. of acid alcohol and the resulting green solution concentrated to a given volume. By colorimetric comparison with a standard green solution, the quantity is indicated as +, ++, or +++.

To determine urobilin and urobilinogen: To 10 c.c. of duodenal contents are added 10 c.c. of Schlessinger's solution, the whole thoroughly shaken and allowed to filter. The filtrate should be slightly alkaline; if not, a drop or two of a weak ammonia solution are added. The filtrate will in the presence of urobilin show a more or less pronounced green opalescence. To 10 c.c. of this filtrate are added 1 c.c. of Ehrlich's benzaldehyd solution.[9] In the presence of urobilinogen a red color will develop. This is allowed to stand in a dark place for fifteen minutes, and is then examined by the spectroscope (Schneider).

TABLE 7.—Biliary Pigments

Cases	Duodenal Contents			Urine		Feces
	Bilirubin	Urobilin	Urobilinogen	Bilirubin	Urobilin	Urobilinogen
Schneider's						
1	+++	+++	+++	0	+	
2	+++	2,000	1,800			
3	+++	4,000	2,800	0	1,000	
4	+++	2,000	1,200	0	1,000	+++
5*	+++	2,300	2,500	..	1,000	+++
Authors'						
1	+++	+++	+++	0	+	+++
2	+++	+++	+++	0	+	+++
3	+++	+++	+++	0	+	+++

* Urobilinogen in urine 100.

We applied these tests qualitatively and confirm Schneider's results (Table 8).

An examination of the *urinary nitrogen partition* was made in each case. In general the results were negative except that the oxyproteic acid nitrogen fraction was increased to twice the normal, a condition frequently met with in carcinoma and in syphilis. The results will be found in Table 8.

8. Schneider: Arch. Int. Med. **17**:32, 1916.

9. Para-dimethylaminobenzaldehyd, 2 gm.; acid hydrochloric, 15 c.c.; water, 15 c.c.

TABLE 8.—URINARY NITROGEN PARTITION

Case	Volume, C.c.	Total N. Gm.	Urea N, per Cent.	Ammonia N, per Cent.	Uric Acid N, per Cent.	Purin N, per Cent.	Creatinin N, per Cent.	Oxy-Protein N, per Cent.	Rest N, per Cent.
1	1,470	16.52	79.3	4.2	2.7	2.1	2.8	3.9	5.0
2	1,625	9.35	81.6	3.7	3.1	2.7	3.1	4.2	1.6
3	960	12.17	82.5	3.4	2.9	2.5	2.6	5.3	0.8

Study of Kidney Function. — The phenolsulphonephthalein test was tried twice on each case. The results, in general, did not indicate any deviation from the normal. It is always advisable, when one obtains a low phenolsulphonephthalein output to repeat the test for confirmation; for, we have found in numerous instances that a low output may only indicate a faulty technic rather than a poor renal function. Table 9 shows the results obtained in the three cases.

TABLE 9.—PHENOLSULPHONEPHTHALEIN OUTPUT

Case	Test Number	Per Cent. First Hour	Per Cent. Second Hour	Per Cent. Total
1	1	25	15	40
	2	48	40	88
2	1	37	30	67
	2	35	34	69
3	1	42	30	72
	2	40	28	68

Examination of the *blood plasma for several nitrogen fractions* (Table 1) showed that there was no increase in the nonprotein nitrogen, urea nitrogen, or uric acid. This is a good evidence of normal kidney function.

The only nitrogen fraction increased in the blood, especially in Cases 2 and 3, is the creatinin, due more to the destructive process present in the body rather than to any failure of the kidney to eliminate this substance. It is remarkable how often syphilitic patients yield a high creatinin figure in the blood.

There was acidosis, to a certain degree, present in all of the three cases of pernicious anemia. The carbon dioxid combining power of the plasma was much reduced, as determined by the Van Slyke method. The H ion concentration is increased, and the carbon dioxid of the alveolar air was 4.4, 4.7, and 4.6 in the three cases, respectively.

HISTORY OF THE CASES

Case 1.—Jewish woman, 45 years old, married; no children; four miscarriages; husband well. A Wassermann[10] was performed on her and twice found positive weakly. When she came to the hospital, she had a red blood count of 720,000, hemoglobin 35 per cent., color index 2, white blood count 6,600, neutrophils 53 per cent., eosinophils 1 per cent., large mononuclears 2 per cent., lymphocytes 44 per cent. There was marked anisocytosis and poikilocytosis. The coagulation time was 12.5 minutes; blood platelets 150,000. She remained in the hospital for three months, being transfused a number of times by the citrate method. Salvarsan had no effect. During one of the remissions she left the hospital.

Case 2.—Married salesman; has one child; ill eight months; denies syphilis; had gonorrhea; Wassermann doubtful. Blood: red blood count 1,200,000, hemoglobin 45 per cent., color index 1.8; marked anisocytosis and poikilocytosis; normoblasts present; platelets 185,000; coagulation time 11 minutes.[11] The patient was transfused several times, but did not rally, and died. No necropsy.

Case 3.—Married woman, aged 42; two children; ill one year. Blood: Wassermann negative; red blood count 1,400,000, hemoglobin 42 per cent., color index 1.3, blood platelets 210,000, coagulation time 13.5 minutes; white blood count 7,200, polynuclears 62 per cent.; many normoblasts. The patient was transfused several times, but left the hospital during a remission.

CONCLUSIONS

1. A study of the chemistry of three cases of pernicious anemia is presented.

2. The blood analysis shows a lessened specific gravity of the serum, reduction of the protein content, an increase in the ash and lime content, and a normal fat, cholesterol and glucose percentage.

3. There is complete anacidity present in the stomach, an increased residuum, and absence of pepsin, resembling the gastric picture present in cases of carcinoma ventriculi.

4. The Wolff-Junghans test is negative.

5. Intestinal digestion is disturbed. The fecal bulk is much increased, and the nitrogen lost in the feces above normal. The fat in the feces is normal.

6. Intestinal putrefaction, as evidenced by increased ethereal sulphate output, is present. There is a state of suboxidation — the neutral sulphur fraction is increased.

7. The pancreas functionates normally, as evidenced by enzyme examination of duodenal contents and feces.

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10. It has been the experience of one of us (Kahn) that pernicious anemia patients very frequently have a positive Wassermann test.

11. The coagulation time was determined by collecting a few drops of blood from an arm vein by means of a needle into a flat bottom test tube. By gently tilting the tube occasionally, one can determine the coagulation time very exactly.

8. There is a deficiency in the hepatic detoxication function as shown by the sulphoconjugation test. The glycogenic and ureogenic functions of the liver are normal.

9. The excessive hemolysis of pernicious anemia is attended by both a pleochromie and a urobilinocholie. In this regard we corroborate Schneider's experiments.

10. There is an increased elimination of oxyproteic acid nitrogen in the urine in cases of pernicious anemia; the other urinary nitrogen fractions being normal.

11. The renal function is normal as evidenced by the phenolsulphonephthalein test, and the blood nitrogen partition.

12. The creatinin in the blood is increased.

13. Acidosis was present in the cases examined, as determined by the carbon dioxid combining power of the blood plasma, the H ion concentration of the blood, and the carbon dioxid of the alveolar air.

The following references may also be consulted:
Addis: Archives Int. Med. **15**:413, 1915.
Abderhalden: Ztschr. f. Biol. **21**:483, 1900.
Asher and Grossenbacher: Biochem. Ztschr. **17**:78, 1909.
Austin and Pearce: J. Exper. Med. **20**:122, 1914.
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Hurwitz: Bull. Johns Hopkins Hosp. **26**:235, 1915.

TOXIN FORMATION BY A VARIETY OF B. BOTULINUS WHEN CULTIVATED AEROBICALLY UNDER VARIOUS CONDITIONS: ITS POSSIBLE PRODUCTION IN THE ANIMAL BODY*

L. P. SHIPPEN, M.D.
Bacteriologist (Miscellaneous Division), Bureau of Chemistry, U. S. Dept. of Agriculture
WASHINGTON, D. C.

Bacillus botulinus was first isolated by van Ermengem[1] who recovered it from meat that had produced an epidemic of botulism. He demonstrated that a soluble toxin secreted by the bacillus in the food was responsible for the condition and that the organism itself had no pathogenic power. That is to say, the bacillus was unable to produce the toxin under the conditions existing in the body.

The bacillus described by van Ermengem was a motile, sporulating anaerobe. This organism fermented dextrose, but did not coagulate milk. It required the presence of peptone in a neutral or slightly alkaline medium for toxin production. At temperatures above 30 C. toxin formation was inhibited. (This latter fact alone explained its lack of pathogenicity in animals having a temperature of 37 C.)

Although an obligatory anaerobe when in pure culture, van Ermengem and others showed that *B. botulinus* produced its toxin in the presence of oxygen when grown in symbiosis with certain aerobes.

Rabbits, cats, guinea-pigs, mice and monkeys are susceptible to the toxin. Chickens and frogs are not affected.

The symptoms produced are not those usually seen in a gastrointestinal intoxication. After an incubation period of from twenty-four to forty-eight hours there arise symptoms referable to the action of the toxin on the nervous system. In animals death is preceded by general motor paralysis and dyspnea, with secretion of mucus from the nose and mouth.

Postmortem, there is seen a general hyperemia of the organs with parenchymatous degeneration and minute hemorrhages. Amongst other changes, the muscles of the heart show signs of degeneration.

The work of van Ermengem has been confirmed in numerous instances. Recently there have been described in this country cases which vary somewhat from the original description.

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* The latter part of this paper consists of preliminary notes not intended originally for publication. Further work along these lines has been postponed indefinitely, and for this reason it is thought best to publish what has been accomplished at the present time.

1. Kolle and Wassermann: Handbuch der path. Mikroorganismen, Ed. 2, 4:922.

Wilbur and Ophüls[2] reported an epidemic at Stanford University which apparently was caused by canned beans. That is to say, the toxin was produced in the absence of animal protein. Postmortem, they described a condition of thrombosis in the blood vessels at the base of the brain. In both of these points the epidemic varied from the original. No organism was isolated.

Later Dickson[3] experimented with two strains of *B. botulinus,* one obtained from the New York Museum of Natural History, the other from Zinsser of Columbia. He concluded that "animal protein is not essential for the development of the toxin of botulism," that "an acid reaction of 3.2 per cent. to phenolphthalein does not prevent the formation of toxin," that "the toxin produces some disturbance of the circulatory system which leads to hyperemia and hemorrhages in the meninges and in the central nervous system, and to thrombosis in the arteries and veins of the meninges and of the central nervous system" and that "the lesions in the nerve cells are not due to a specific action of the toxin on the cell protoplasm, but they are secondary to disturbances of blood supply."

In another instance Nevin[4] isolated from cottage cheese that had caused three deaths an organism closely resembling *B. botulinus* of van Ermengem. In this epidemic the clinical diagnosis was not made, and no organism capable of producing the symptoms was recovered postmortem.

The organism isolated from the cheese by Nevin was diagnosed as *B. botulinus,* but differed from this organism in the following particulars: It formed its toxin in a medium containing only the peptone of the meat, from which the medium was made, and it produced this toxin readily at 37 C.

These are very important variations from the organism of van Ermengem. Indeed, the production of toxin at 37 C. renders its nonpathogenicity a matter of doubt. Further, the fact that *B. botulinus* produces its toxin under aerobic conditions when cultivated with certain aerobes, and that according to Dickson[3] a reaction of +3.2 does not entirely inhibit its production, and that animal protein is not essential, would indicate that toxin formation by *B. botulinus* might occur under conditions not generally thought possible.

EXPERIMENTAL

In the following experiments an attempt was made to study toxin production under unfavorable conditions such as might occur within and without the animal body in nature.

2. Wilbur and Ophüls: Archives Int. Med. **14**:589, 1914.
3. Dickson: Botulism, An Experimental Study, J. A. M. A. **65**:492, 1915.
4. Nevin, Mary: A Study of Cheese Causing Three Fatal Cases of Botulism. Read in abstract before the Conference of The American Public Health Association, Rochester, N. Y., Sept. 8, 1915.

The strains used by Dickson originally from Zinsser and the New York Museum of Natural History were found to have lost their toxin forming power as noted by Nevin. But the Nevin strain produced a powerful toxin and was used throughout the work.

The organism was found to differ somewhat from *B. botulinus* of van Ermengem in its behavior towards lactose. In mediums containing this sugar *B. botulinus* grows with difficulty, according to van Ermengem, who recommends dextrose as an enriching substance. The strain isolated by Nevin, on the contrary, grew very profusely in mediums containing from 1 to 2 per cent. lactose. In the hands of the writer it did not ferment this sugar with production of acidity or gas in spite of the fact that it possessed the power of acidifying and peptonizing milk. But according to Nevin it does possess this power.

In dextrose mediums it produced fermentation, as does *B. botulinus* of van Ermengem. The acidity of this reaction supposedly is not favorable to the toxin. Since growth without acidity occurred in the presence of lactose this substance was used instead of dextrose. Anaerobically, in sugar-free meat extract medium, the organism failed to grow.

One per cent. lactose agar of a neutral reaction was employed for the stock cultures and for testing the power of the organism to multiply under aerobic conditions. For toxin production it was cultivated in a broth adapted from that advocated by Rideal and Walker for testing disinfectants. This medium has the following ingredients:

	Gm. or c.c.
"Liebig's" extract of meat	20
Peptone (Witte's)	20
Salt	10
Distilled water	1,000

To this was added 20 gm. of lactose and the product was then made alkaline to the point of —0.5. This medium will be referred to as L. R. W. broth.

Tests for Toxicity.—The toxicity of the Nevin organism was first tested against rabbits subcutaneously. In the preliminary experiment a very large dose was used. Two c.c. of a ten-day culture in L. R. W. broth grown anaerobically at about 30 C. in the dark were given by subcutaneous injection to a rabbit weighing 1,465 gm. Nineteen hours later the animal was found moribund, with all the muscles relaxed and saliva dribbling from the mouth. In another hour death occurred. The postmortem was performed immediately, and the following lesions were observed:

There was no gas in the tissues; serous exudate with some hemorrhage at the seat of inoculation; peritoneum and mesentery injected and organs, in general, hemorrhagic with the exception of the spleen, which appeared normal. The diaphragm and wall of the heart were likewise injected. Smears from the exudate, liver, spleen and heart's blood showed no organisms except for a few doubtful coli-like bacteria in the exudate. The organism was recovered in

pure culture by anaerobic methods from the heart's blood, liver and spleen. The culture from the heart's blood was retained and used in some of the experiments to be described, as it was believed that its toxin producing power might have been increased. It will be referred to as the H. strain.

The toxicity of the same strain when given per os was now tested. One c.c. of a four-day old culture grown as in the preceding experiment was placed on a little uncooked oatmeal, which completely absorbed the liquid. This preparation was given to a rabbit weighing 2,080 gm. The following morning the animal was observed to crawl instead of hopping in the usual fashion. No other symptom was noticed. On the second day the rabbit was unable either to hop or crawl, and died about fifty-four hours after eating the oatmeal. Postmortem the following points were observed:

There was marked injection subcutaneously, in mesentery, around the gut, on the stomach wall and in the diaphragm. The liver and spleen were congested and enlarged. The kidneys appeared normal. The bladder was full. Two lobes of the lungs were markedly congested. The heart was swollen and firm, and the vessels congested. There was no increase of fluid within the pericardium. Anaerobic cultures in L. R. W. broth from the heart, liver and spleen failed to show any growth.

One c.c. of the H. strain, made directly from the heart's blood of the first rabbit after three days' growth under similar anaerobic conditions, was next placed on some oats in a pan and given to a rabbit weighing 1,305 gm. The animal ate very little of the oats and since most of the culture trickled to the bottom of the pan only a very small portion of the broth could have been consumed. Yet on the morning following, the rabbit showed symptoms of weakness of the hind legs and lack of control of urine. In the afternoon of the same day the animal died, a little over twenty-four hours after ingestion of the poison. No postmortem was performed.

Finally, 0.1 c.c. of a five-day anaerobic culture of the original strain was placed in 1.0 c.c. of water, poured on a little oatmeal and given to a rabbit weighing 1,470 gm. The animal at first refused to eat this food, which was not consumed until the middle of the following day. No symptoms were apparent during the next twenty-four hours, but on the succeeding morning, about forty-five hours after the ingestion of the toxin, the rabbit appeared moribund, lying quietly on his side and dying soon.

The postmortem revealed marked injection of the blood vessels subcutaneously, around the gut, in the diaphragm, and on the heart wall. The spleen, liver and kidneys were markedly congested, but the lungs only moderately so. Slight hemorrhage was observed within the pericardium. Anaerobic and aerobic cultures were negative.

The importance of these experiments in which the toxin was given per os lay in the smallness of the lethal dose. For the toxin of *B. botulinus* of van Ermengem produces death under similar conditions only in doses of 5 to 10 c.c.

When tested against the cat the organism was not found to possess markedly toxic power, either per os or subcutaneously. One c.c. of a four-day anaerobic culture of the H. strain was given by stomach tube to a cat weighing 2,000 gm. The next day the animal's hind legs seemed a little stiff and in the afternoon it refused meat. The following morning a trembling in the tail was noticed, but in the afternoon the appetite returned, and from that time on no further symptoms were noticed.

To another cat was given a piece of meat in which had been injected 1 c.c. of a twelve-day anaerobic culture. This animal developed no symptoms.

A third cat was injected subcutaneously with 1 c.c. of a two-weeks-old culture of the same strain. On the following morning marked weakness in the hind legs was observed and the animal evidently preferred the dark. However, meat was not refused, and the symptoms gradually disappeared in the course of four days.

Another cat injected subcutaneously with 1 c.c. of a four-day anaerobic culture of the original strain failed to develop any symptoms during the first week, at the end of which period it was discarded.

The resistance of the cat to doses of 1.0 c.c. given subcutaneously differs from the reaction of the animal to the toxin produced by the strain of van Ermengem which caused death in quantities of from 0.1 to 1.0 c.c. But it should be noted that according to Nevin[4] the toxin of the organism produced typical symptoms in kittens.

In regard to chickens and frogs, the organism of Nevin when fed per os acted according to the descriptions of *B. botulinus* of van Ermengem. In doses of 5 c.c. neither strain had any noticeable effect on two chickens fed with corn meal moistened with the cultures.

One frog was injected with 1.0 c.c. of the original strain, and another frog was placed in a jar containing 1.0 c.c. of the culture mixed with a little water. Neither developed any symptoms.

A third frog was placed in a jar containing 2 c.c. of the H. strain. It, likewise, failed to show any bad effects during a period of a week.

Finally[5] the toxicity of the organism for horses when given per os was tested. For this purpose a four-day anaerobic culture of the H. strain in L. R. W. broth was used. Ten c.c. of this preparation were poured on some oats and given as feed to a young colt. Opportunity for close study of any symptoms was not available, but up to 5 a. m. of the second day after ingestion of the toxin, no symptoms were observed by the stable attendants. Three hours later, at 8 a. m., about forty-four hours after eating the oats, the animal was found dead. The postmortem was performed the same morning by Dr. Buckley and the following points noted:

Engorgement and inflammation of the jejunum and lungs; slight hemorrhages on the heart wall; engorgement of the lymph gland near the spleen and slight injection of the meninges. Transfers from the heart's blood, liver and spleen kept under anaerobic and aerobic conditions were sterile.

Experiments in Forage Poisoning.—The fatal result following the ingestion of the culture by the horse confirmed the possibility of the Nevin organism being the causative factor in forage poisoning and led to an attempt to reproduce this disease by treating forage with preparations cultivated under aerobic conditions similar to those found in nature. At the same time an effort was made to demonstrate the extent to which this organism might be cultivated in symbiosis with an aerobe in the presence of oxygen and still retain its toxin producing power.

Since the food supposed to be responsible for forage poisoning is frequently heavily seeded with yeasts, a variety of this species was chosen as symbiotic organism. It possessed the following characteristics:

Milk: Not changed.
Gelatin: Not liquefied.
Broth: Growth in form of sediment; no turbidity.
Dextrose: Acid and gas.
Lactose: No acid; no gas.
Saccharose: Acid and gas.
Pathogenicity: No effect on rabbits when injected subcutaneously in doses of 5 c.c.,, or when given per os.

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5. The data in regard to the effect of cultures on horses has already been published by Dr. John S. Buckley of the Bureau of Animal Industry, in the Journal of American Veterinary Medical Association, March, 1917, New Series, Vol. 3, No. 7. His paper was first presented at the meeting of the A. V. M. A., Detroit, Mich., Aug. 21-25, 1916. For reasons apparent in the conclusions, this data is now reprinted. This part of the work was performed in order to demonstrate whether or not the organism may be the causative factor in the disease of horses commonly known as forage poisoning.

This yeast was grown on wort agar, and whenever transfers were made to preparations containing the Nevin organism a loop was used, since it had been previously determined that the latter organism grew irregularly when small quantities of the yeast were collected with a wire. The culture employed was that recovered from the heart's blood of the first rabbit, and which had been used in the case of the horse.

The cultures were kept under aerobic conditions on top of the incubator covered by a black cloth to protect them from the light. The temperature ranged from 25 to 32 C.

The mediums on which the mixed cultures of the Nevin organism and the yeast were grown consisted of ordinary 1 per cent. lactose agar slants and the L. R. W. broth already described. A trace of dextrose was probably present in both instances.

Transfers were first made from an anaerobic lactose agar preparation of the Nevin organism to two tubes of the L. R. W. broth. To one tube was added a heavy inoculation from a wort agar culture of the yeast.

After three days turbidity appeared in the preparation containing both bacillus and yeast, and a hanging drop showed the presence of motile bacilli. The yeast was present in the sediment, but only in very small numbers in the supernatant fluid. In the tube inoculated with the anaerobe alone no turbidity appeared during ten days, and no trace of the organism could be observed microscopically, indicating that no aerobic contamination existed in the anaerobic culture from which the aerobic preparation was made.

The mixed culture was now transferred to two lactose agar slants, and to one of them was added a loopful of the yeast.

After forty-eight hours a few colonies of the yeast were observed on the slant to which no additional yeast had been conveyed. Microscopically, yeast cells, but no bacilli, were seen. The other tube to which had been carried a heavy transfer of yeast showed an irregular growth in which appeared microscopically a few motile bacilli, without spores, mingled with the yeast cells.

This preparation was now transferred to two slants of the same medium over one of which, as in the previous case, a loopful of the yeast was smeared. After forty-eight hours the simple transfer showed microscopically many yeast cells and a few nonmotile bacilli without spores. The other tube to which the yeast had been conveyed showed the same picture except that motility was observed in the bacilli.

The latter preparation was transferred to a fresh tube of lactose agar with the addition of yeast as in the previous cases. No microscopic observations were made. After three days this culture was again conveyed to two slants of lactose agar, and to one of these yeast was added. At the end of forty-eight hours both these tubes showed irregular, heaped-up growths. Microscopically, there were seen numerous bacilli along with a few spores in addition to the yeast cells.

This process was repeated, and in forty-eight hours both the simple transfer and the culture with additional yeast showed microscopically equally numerous motile bacilli mixed with the yeast. Therefore, the further addition of yeast was not considered necessary, and the simple transfer was selected and conveyed by means of a loop to a fresh lactose agar slant.

In this preparation after three days the motile bacilli were found to be present in small numbers. From this tube another transfer was made, and again in three days the same picture was seen, except that some spores were noted as well.

This culture was transferred to a final lactose agar slant and then the medium was changed in order that the organism might be given an opportunity to produce its toxin in a liquid. After two days' cultivation the final lactose

agar preparation was carried with a loop along with a fresh supply of yeast to L. R. W. broth. Then after four days, and again after three days, transfers were made, with addition of yeast, from L. R. W. broth culture to L. R. W. broth.

In this latter preparation turbidity and sedimentation appeared in forty-eight hours. On the third day this increased, and microscopically in the supernatant fluid were seen motile bacilli with subterminal spores. In the sediment were yeast cells.

Thirty-two days had now elapsed since the first aerobic transfer and during this period the bacillus had been successfully cultivated for seventeen days on lactose agar slants by means of eight transfers.

The toxicity of the last broth culture of the Nevin organism and the yeast was next tested:

One c.c. of this culture was given by stomach tube to a rabbit weighing 1,760 gm.; 1 c.c. was injected into a piece of meat and given to a cat to eat.

No symptoms were noticed in the cat during a period of a week after ingestion of the food, but the rabbit began to show slight sluggishness after three days, and on the succeeding day was found dead in his cage.

The postmortem showed the usual findings somewhat intensified and there was also observed a marked congestion of both lungs, with the exception of part of one lobe, and a hemorrhagic pericarditis with blood clots. The surface of the kidney presented a curious pitted appearance. Smears and anaerobic and aerobic cultures from the heart's blood, liver and spleen were negative.

In the meantime the aerobic mixed culture was carried on in L. R. W. broth as formerly. That is to say, a fresh transfer with the addition of the yeast was made every three or four days. After three such transfers, at the end of a further period of twelve days, 1 c.c. of a three-days' culture was given by stomach tube to a rabbit weighing 2,180 gm. The next morning the animal was found dead. The postmortem was performed on the following day and showed a marked hemorrhagic congestion of the lungs and bloody fluid in the pericardium, with injection of the blood vessels of the heart wall and duodenum. Postmortem decomposition had set in and masked the findings.

At the end of another forty-eight hours 1 c.c. of the next transfer was dropped on a little oatmeal and given to a rabbit weighing 2,265 gm. The oatmeal completely absorbed the fluid and was entirely consumed by the following morning. Symptoms of weakness developed on the fourth day and the animal ceased to eat, although his jaws were observed to move as if chewing the food. After another period of two days this attempt also ceased, and during the next week the food placed in the rabbit's cage remained untouched. No symptoms other than those of weakness were observed. Then improvement set in and the animal was discarded after another week had elapsed. At this time he was able to eat and seemed apparently normal.

The next transfer was used to test the toxicity of the preparation for donkeys: 0.2 c.c. of the culture when three days old was placed in about 10 c.c. of sterile saline solution and given to a donkey by pouring on a little bran. The bran was consumed within two hours after being treated with the culture. As a control, 1 c.c. was placed on some uncooked oatmeal and given to a rabbit weighing 1,520 gm.

The rabbit developed no symptoms till the end of the second day, when a retraction of the neck was noted. On the following morning he was found dead. Postmortem, the animal showed the usual general injection of the blood vessels, and as in the first two rabbits, the lungs were congested. Hemorrhage had occurred in the pericardium. The kidney and liver were congested and the spleen was enlarged and of a pulpy consistency. Neither the Nevin organism nor the yeast could be recovered by anaerobic or aerobic methods.

The donkey became sick at the end of four days after the feeding. At this time he was seen by the caretaker of the stable to be lying down. The writer was not able to see the animal until another forty hours had elapsed, six days after ingestion of the culture. At this time the donkey was prostrate, breathing heavily, with dilated nostrils, from which a mucous fluid was dribbling. The animal was too weak to rise or walk unaided. When raised, he took a few steps supported by several men. He was then pushed back in the stall, where, after standing for ten or fifteen minutes, he fell. No symptoms indicating paralysis could be observed. On the following morning the donkey was dead. Opportunity for a postmortem did not occur before the next day, when decomposition completely masked any lesions that migh have been present. No cultures were taken. The temperature chart for the first four days follows:

TEMPERATURE, DONKEY 1

Date	A. M.	Noon	P. M.	
Feb. 15 '16 p. m.	Culture ingested.		98.2	
Feb. 16	97.2	97.0	97.6	
Feb. 17	97.2	96.4	98.6	
Feb. 18	97.2	96.6	99.0	
Feb. 19	96.0	97.8	96.8	marked ill-
Feb. 20				ness
Feb. 21				
Feb. 22	Death.			

In the meantime the mixed culture of *B. botulinus* and the yeast had been carried on in the usual manner by transferring every third day to L. R. W. broth, with the addition of yeast at each transfer. After two such transfers 1.0 c.c. was given on oatmeal to a rabbit weighing 1,460 gm. as a control, and 0.5 c.c. was given on bran to a second donkey.

The rabbit developed a slight weakness in the hind legs on the following day and was found dead the next morning, less than forty-eight hours after eating the food. The postmortem was performed at once, but decomposition had already set in. However, injection of the blood vessels in the subcutaneous region, in the diaphragm and around the wall of the duodenum could be made out. The lungs showed areas of congestion. Aerobic and anaerobic cultures from the heart's blood were negative.

The donkey on the second morning after eating the infected bran, that is to say, after a lapse of about forty-two hours, was observed to paw the earth and tremble in a manner to indicate the possible existence of some intestinal colic. No other symptoms were manifest. On the evening of the same day he seemed sluggish, but presented no definite signs of illness. However, on the morning of the third day, about sixty-six hours after eating the bran, he was found with the tongue completely paralyzed, protruding about 3 inches or more from the mouth. Food and drink were refused. The pulse was about 30 and somewhat weak. The temperature was 99. The sluggishness was more marked than on the preceeding evening, but there were no signs of paralysis other than those evident in the tongue. At 2 p. m. of the same day the animal was noticed by the watchman, standing in his stall, the condition apparently unchanged. At 4 p. m. he was seen by the writer. At this time the donkey was lying down, too weak to rise without aid. When forcibly lifted, he was unable to stand alone, and when put back on the ground lay quietly, with apparent consciousness of his surroundings. The tongue still protruded, but no other signs of paralysis could be noted. About midnight the donkey died, in the neighborhood of three days and eight hours after eating the bran, and fifteen hours after the first serious symptom, paralysis of the tongue, was observed. The temperature chart, taken by the stable attendant, follows:

TEMPERATURE, DONKEY 2

Date	A. M.	Noon	P. M.
Feb. 21 '16 p. m., culture ingested.			99.2
Feb. 22	98.0		99.0
Feb. 23	98.0		98.0
Feb. 24	99.0	97.8	97.0 Death 12 M.

The postmortem was performed by Dr. Buckley about ten hours after death had occurred, before any signs of decomposition had set in. The following points were noted:

Subcutaneous vessels not markedly injected.

Stomach: Vessels in outer wall injected. Inner wall shows areas of shallow erosions. Mucous membrane is quite pale and coated with mucus.

Duodenum: Outer wall shows areas of hemorrhage in patches. On inner wall are punctate and larger areas of hemorrhage.

Jejunum: Outer wall like that of duodenum, except that areas of punctate hemorrhage are present in addition to the larger patches. The inner wall shows a condition intermediate between the duodenum and ileum.

Ileum: Outer wall like that of jejunum, but lesions are more marked. On inner wall is seen a condition of diffuse hemorrhagic enteritis.

Cecum: Outer wall normal. Inner wall shows numerous punctate hemorrhages.

Colon: First part reddened on outside. Stained on inside. Second and third parts normal and full of feces.

Mesentery: Larger vessels are injected.

Spleen normal.

Liver slightly congested.

Kidney markedly congested.

Diaphragm, no marked injection.

Lungs markedly congested.

Heart wall, no points of hemorrhage.

Pericardium normal.

Bladder full but not distended.

Cerebrospinal fluid, normal in quantity at junction of head with body.

Cultures were made from the heart's blood, liver and spleen in L. R. W. broth, on plain agar and on wort agar. The L. R. W. broth cultures were placed under anaerobic conditions. The others were kept aerobically. All were kept at a temperature of between 25 and 30 C. On none did any growth develop.

These experiments indicate that the organism, although in pure culture an obligatory anaerobe, may be cultivated in mixed culture with a yeast under aerobic conditions such as exist on an aerobic slant agar preparation.

Effect of Light on Toxin Production.—The effect of light on the production of toxin was next studied. The medium employed consisted of 2 per cent. lactose R. W. broth of reactions varying from neutral to about +2. Traces of dextrose were present. About 10 or 15 c.c. were kept in each tube. A tube of this medium was inoculated from a dextrose agar preparation of the same strain used in the previous experiments, and with the yeast from a twenty-four-hour wort agar culture. Other tubes were inoculated with simple transfers of the two organisms. All the preparations were kept on the desk in front of a window at temperatures ranging from 20 to 30 C. The window faced the North, and the sunlight did not fall on the cultures.

In the tube containing the Nevin organism alone no growth developed. In the tube to which the yeast had been transferred there occurred a sediment without turbidity. Microscopically only yeast cells could be demonstrated. It may, therefore, be inferred that the cultures used contained no aerobic contamination capable of growing in broth.

In the tube containing both anaerobe and yeast there developed in forty-eight hours the usual turbidity, sediment, and foul odor. The hanging drop revealed the presence of motile bacilli with subterminal spores, and yeast cells.

After the culture was five days old 1 c.c. was dropped on some uncooked oatmeal and given to a rabbit weighing 1,620 gm. This was consumed by the following morning. Twenty-four hours later the rabbit ceased to eat. After another interval of forty-eight hours the animal was found dead. From the condition of the body death probably occurred between forty-eight and seventy-two hours after eating the oatmeal. Postmortem there was observed the decomposition that was to be expected and the usual injection of the blood vessels and engorgement of the liver. The lungs were markedly hemorrhagic, only a few areas being unaffected.

The culture was kept alive by transferring every five or ten days to fresh L. R. W. broth with the addition of yeast. The tubes were kept on the desk in the light under aerobic conditions. After being thus grown for twenty-three days a five-day culture was given in dose of 1 c.c. by stomach tube to a rabbit weighing 1,785 gm. No symptoms developed until the afternoon of the fourth day after the feeding. Then the animal was observed to make continual efforts to swallow without eating. The following morning this persisted and in addition the neck was retracted. There was no paralysis of the limbs. At 3:30 p. m. the rabbit was found dead and stiff, with neck retracted.

The postmortem showed a tumor above the umbilicus connecting with the region of the spine and a hemorrhagic fibrinous exudate beneath the skin. The peritoneum, mesentery, diaphragm and heart wall were injected. The heart wall was congested. The lungs were normal, except for one small area of hemorrhage. The liver was congested. The spleen was normal. Cultures made anaerobically and aerobically from the heart's blood were negative.

After being grown in the light on the desk for two and one-half months, with transfers every five or ten days, 5 c.c. of a fourteen-day culture were given by stomach tube to a rabbit weighing 1,890 gm. No symptoms were observed the following day. At 4 p. m. the rabbit ate part of a carrot. The next morning, less than forty-eight hours after ingestion of the culture, it was found dead, with eyes open, lying on the side, without any signs of a struggle. The carrot given the previous afternoon was half consumed. Postmortem, there was seen the usual injection, and in addition there was one large area of hemorrhage on the upper gut. The lungs were engorged. The heart swollen, firm, congested. The vessels of the diaphragm were markedly injected. The spleen and kidney were about normal.

These experiments indicate that exposure to daylight for two and one-half months does not inhibit the toxin production to any marked degree.

Effect of Heat on the Toxin.—The effect of heat on the toxin and on the toxin-forming power of the bacillus was now taken up.

A five-day aerobic mixed culture in L. R. W. broth grown at temperatures under 26 C. was selected. As a control, 1 c.c. of this culture was given by stomach tube to a rabbit weighing 1,785 gm. Then the preparation was heated to from 65 to 70 C. for twenty minutes, and 2 c.c. of this were injected subcutaneously into a rabbit weighing 2,460 gm. The control developed symptoms four days later and died on the following day. The postmortem showed the usual findings. The other rabbit failed to develop any symptoms during eight days following the inoculation.

This experiment was repeated, with the variation that the heated culture was given per os instead of subcutaneously, and the period of observation lengthened in order to determine the ability of the spores to develop in the rabbit's intestines. Ten c.c. of a thirteen-day mixed culture grown aerobically in L. R. W. broth at a temperature of 30 C. in the incubator and heated to from 65 to 70 C. for twenty minutes were given by stomach tube to a rabbit weighing 2,400 gm. At the end of six weeks no symptoms had developed and the rabbit weighed 2,335 gm.

These experiments confirmed those of many other workers in regard to the destruction of the toxin by moderate amounts of heat, and indicated that the spores of the organism were not pathogenic when given subcutaneously or per os.

Regaining Toxicity After Heating.—To demonstrate the possibility of a heated culture regaining its toxicity, the following experiments were undertaken.

A five-day mixed culture grown aerobically on the desk at temperatures below 25 C. was heated to from 65 to 70 C. for twenty minutes. As a control, 2 c.c. of this preparation were placed on raw oatmeal and given to a rabbit weighing 1,980 gm. The food was not entirely eaten till three days had elapsed, but since no symptoms developed in the ensuing four days, and since other experiments had shown that heated cultures were not toxic, it may be concluded that in this case as well the heat applied had rendered the toxin harmless.

Since in the original heated culture the yeast had been killed, the remaining spores of the anaerobe could not be expected to develop aerobically and produce toxin without the addition of a fresh supply of yeast. Accordingly, immediately after cooling, a fresh transfer of yeast was made to the culture and the preparation placed on the desk. After a week had elapsed, during which time the temperature ranged under 26 C., 1 c.c. of this preparation was placed on oatmeal and given to the same rabbit, whose weight was now 1,970 gm. The food was consumed during the next forty-eight hours, but no marked symptoms developed during the six days following the giving of the culture. To determine its toxicity finally, 2 c.c. of this same preparation, thirteen days having passed since the heating, were injected subcutaneously into the rabbit at 3 p. m. The following morning the animal was found dead, lying stretched out in the cage with no evidence of convulsions having occurred. Death had ensued in from twelve to eighteen hours.

Postmortem there was observed a serofibrinous exudate beneath the skin, without noticeable hemorrhage. There was some injection of the peritoneum and mesentery. The heart was swollen, but not otherwise abnormal. The lungs showed no congestion. The diaphragm was thickened, and the vessels injected. The liver was congested, the spleen markedly enlarged and congested, the kidneys congested, the bladder full and the vessels injected. Cultures from the heart's blood were negative.

On account of the failure of the control rabbit to eat the oatmeal immediately on being offered, and on account of too short a time being allowed for observation, it is not possible to draw any very definite conclusions from this preliminary experiment. However, it would indicate that contaminated food heated sufficiently to destroy the toxin but not to kill the spores may again become toxic.

Effect of Drying on Toxicity.—To test the effect of drying on the toxin-producing power of the bacillus a small piece of filter paper was saturated with a seven-day mixed culture made in L. R. W. broth. This was placed in a sterile test tube and allowed to dry out on the desk for twenty-two days. Then it was transferred to a tube of L. R. W. broth along with additional yeast. This preparation was placed in the incubator at 30 C. After four days, turbidity and odor were noted.

When the culture was seven days old 2 c.c. were given by stomach tube to a rabbit weighing 1,505 gm. No symptoms appeared the following day. On the second day no observations could be made. On the third day, seventy-two hours after ingestion, the animal was found dead. No postmortem could be performed. Apparently drying for twenty-two days fails to destroy the toxin-producing power of the organism.

Toxin Production at Different Temperatures and Reactions.—According to Dickson, *B. botulinus* may produce its toxin in medium of reaction of over +3,

and according to Nevin the organism isolated by her produces toxin at a temperature of 37 C. To test these observations broth was made according to the following formula:

	Gm. or C.c.
"Liebig's" extract	5
Armour's peptone	10
Salt	5
Water	1,000

Different lots were titrated to 0, +1, +2, +3 and +4 cold, and 10 c.c. measured into tubes. After autoclaving, a tube of each titration was inoculated from a mixed culture of the Nevin organism and yeast grown four days in L. R. W. broth at 30 C. and an additional transfer of yeast was added. The preparations were placed in the incubator at 38 C. In twenty-four hours faint turbidity without odor appeared in the tube of reaction 0. More marked turbidity with odor appeared in tubes of reaction +1 and +2, and no turbidity in tubes of reaction +3 and +4. Since the yeast grows poorly in neutral or alkaline medium and since in pure culture the Nevin organism will not grow aerobically, it was to be expected that a moderate acidity would give the best symbiotic growth.

In seventy-two hours turbidity and some odor was observed in the +3 tube. On examination in the hanging drop, tubes of reactions 0, +1 and +2 showed large motile bacilli, extracellular spores and yeast cells. Tube +3 showed fewer, larger bacilli with intracellular spores and yeast cells, and tube +4 only yeast cells without bacilli.

When the cultures were seven days old a transfer was made from the +3 preparation to a similar tube of +3 broth and additional yeast added. This was likewise placed in the incubator at 38 C. In it there developed turbidity and odor, and when five days old 1 c.c. was taken and given by stomach tube to a rabbit of 1,730 gm. weight. At the same time a further transfer to +3 broth tube was made with addition of yeast. During the next six days no positive symptoms developed in the rabbit, so at the end of this period 5 c.c. of the six-day old culture, made when the first dose was given the rabbit and grown at 38 C., were given to the same rabbit by means of a stomach tube. The following day the animal was sluggish, and in forty-eight hours it ceased to eat. In seventy-two hours, on the morning of the third day after ingestion of the last dose, it appeared very apathetic. The muscles were relaxed and it did not eat. No paralysis was observed. At 3 p. m. the same day it was very weak, the muscles of the abdomen greatly relaxed, and the breathing labored. There was no paralysis. On being placed on the floor it hopped quite energetically, but on being returned to the cage died in convulsions as if from embolus.

Postmortem, the injection in the mesentery around the gut was very marked. The liver and spleen were engorged. The diaphragm was injected. The lungs appeared normal except for one small dark area in one lobe that resembled an infarct. The heart was distended and engorged. Transfers from the heart's blood were negative.

This experiment confirms the observations of Dickson and Nevin in regard to reaction and temperature. It would indicate that in the absence of sugar a weakened toxin is formed in meat extract broth of reaction of +3 at 38 C.

These points do not coincide with the observations of van Ermengem and others in regard to the original strain of *B. botulinus* and indicate that while the organism isolated by Nevin may be a variety of *B. botulinus,* its requirements for toxin production make it theoretically a much more dangerous organism. For since it can produce

toxin at body temperature under aerobic conditions in a medium of +3, it is possible that it might grow in the human intestine, or in the throat in symbiosis with an appropriate aerobe.

That it fails to produce toxin in the intestines of the rabbit has been shown in an experiment already recorded. This may be due to some inhibiting effect of intestinal organisms such as *B. coli* or *Bact. welchii*. In the throat its growth would to a great extent depend on its power to multiply in symbiosis with streptococci, *Micrococcus aureus* and other bacteria commonly found in infections, and to its ability to withstand the inhibiting influences which the body tissues possess.

Opportunity to solve all these questions was not available. To test the ability of the Nevin organism to produce toxin in the presence of *B. coli, Bact. welchii,* and *M. aureus,* the following experiments were undertaken.

Toxicity in Presence of Other Organisms.—In the first series sterile milk was used as a medium. About 10 c.c. were placed in each tube. One was inoculated from an eight-day broth culture of *B. coli* and with a mixed culture of the Nevin organism and yeast grown eight days aerobically at 30 C. in L. R. W. broth. To another tube was transferred the Nevin organism and yeast culture, along with a transfer from a three-day wort agar culture of the yeast. Two other tubes were inoculated with single transfers from the *B. coli* and the yeast culture as controls. All were kept aerobically at 30 C.

In the course of a week the yeast caused no change in the milk. In twenty-four hours *B. coli* produced acidity with slight reduction. In seventy-two hours no coagulation occurred. In thirteen days there was coagulation, with little whey formation, together with some reduction of the litmus and a sweetish odor.

In twenty-four hours the yeast and Nevin organism caused no change. In seventy-two hours there was about 90 per cent. peptonization. After thirteen days there appeared a heavy scum. The odor was very foul.

The yeast, Nevin organism and *B. coli* culture caused a reduction in the litmus in twenty-four hours. In seventy-two hours it produced coagulation with little whey formation. In thirteen days the milk was 50 per cent. peptonized. The odor was sweetish. Here the peptonization would indicate the growth of the Nevin organism, but the lack of foul odor would indicate the opposite.

Hanging drops made from the whey of the yeast and Nevin organism preparation showed bacilli of the general type of *B. botulinus* with subterminal spores. There was no motility. The yeast was present. No explanation of the scum was found unless it was formed by the yeast.

Hanging drops from the whey of the *B. coli,* Nevin organism and yeast culture showed many large and small nonmotile bacteria and many yeast cells. There were no spores and no bacilli of the general morphology of *B. botulinus.* Apparently *B. coli* had inhibited the growth of the Nevin organism. As a final test the two cultures, now evaporated down to about 7 c.c., were given to two small rabbits by stomach tube. The yeast and Nevin organism was given to a rabbit weighing 910 gm. The *B. coli,* Nevin organism and yeast preparation was given to one of 955 gm. weight. The cultures at the time were fourteen days old.

The following morning, twenty-four hours after ingestion, no symptoms were observed in either rabbit. At midday both were well. At 2 p. m. of the same day the rabbit treated with the yeast and Nevin organism preparation was

found dead. The postmortem revealed slight subcutaneous injection. Around the gut this was more marked; the liver was engorged; the diaphragm injected; the upper lobes of the lung showed a hemorrhagic condition; the heart was still beating when examined; it was apparently normal. Cultures made from the heart blood were sterile.

At 4:30 p. m. of the same day the remaining rabbit was lively. At 9 a. m. of the following morning it was found dead and stiff, and had apparently been dead some time. Less than forty-eight hours had elapsed since the ingestion of the culture. Postmortem decomposition had set it. The animal was very rigid. Injection of subcutaneous and mesenteric vessels was present; the liver was engorged; the spleen normal; the heart normal; the upper lobes of the lungs were markedly hemorrhagic.

It is therefore evident, in spite of the Nevin organism not being demonstrable microscopically in the mixed culture, that *B. coli* has not the power of suppressing the growth of the Nevin organism in milk.

The experiment in regard to *Bact. welchii* was not completed by animal testing on account of lack of time. In the preliminary work *Bact. welchii* was first grown aerobically with the yeast in broth, and this was used as a stock culture. Tubes of plain broth of reaction +1, of neutral L. R. W. broth, and of 2 per cent. dextrose broth were inoculated from a twenty-four-hour culture of *Bact. welchii* and yeast, and from an eight-day culture of the Nevin organism and yeast in neutral L. R. W. broth. One set was placed at 30 C., one at 39 C. After twenty-four hours, motile bacilli could be demonstrated in the neutral, L. R. W. and plain broth cultures kept at 30 and 39 C. But in the 2 per cent. dextrose cultures, both kept at 30 and 39 C., no motile bacilli of the morphology of *B. botulinus* could be demonstrated, but only nonmotile fat bacteria of the type of *Bact. welchii*. This would indicate that *Bact. welchii* cannot inhibit the Nevin organism in the presence of lactose alone, or in the absence of sugar, but that it has some inhibitory power in the presence of dextrose. However, the lack of animal tests renders this inconclusive. Yet it is possible that the presence of both dextrose and *Bact. welchii* in the rabbit's intestine is a cause for the nonpathogenicity of the Nevin organism under these conditions.

In regard to the growth of the Nevin organism in symbiosis with *M aureus*, the following experiment was performed.

A tube of plain extract broth of acidity +1 was inoculated from pure agar cultures of *M. aureus* and the Nevin organism and placed at 38-39 C. The *M. aureus* strain selected was one which had produced typical lesions in the rabbit. In twenty-four hours turbidity appeared and in the hanging drop could be seen motile bacilli with subterminal spores. In seven days the hanging drop showed cocci, extracellular spores and nonmotile bacilli of the general morphology of the Nevin organism. Five c.c. of this culture were given by stomach tube to a rabbit weighing 2,419 gm. On the following day no symptoms developed. Two days later, three days after ingestion of the culture, the rabbit was found stiff and decomposed. It had evidently died between twenty-four and forty-eight hours after ingestion. In spite of the decomposition there could be made out marked injection of the vessels in the subcutaneous tissue, mesentery and diaphragm; the liver was engorged; the spleen was somewhat enlarged; the kidneys pitted; the upper lobes of the lungs were markedly engorged; the lower lobes were not affected. No cultures were taken

SUMMARY

The organism isolated by Nevin from cottage cheese that had caused an epidemic diagnosed postmortem as botulism is a distinct variety of the *B. botulinus* of van Ermengem.

It differs from this organism in the following particulars:

It is more toxic for rabbits.

It is less toxic for cats.

It grows and produces toxin at 37 C. and in sugar-free media of acidity of +3.

In the rabbit it produces symptoms and lesions not identical with those described by van Ermengem.

Although stock cultures obtained as *B. botulinus* were found nontoxic, the organism of Nevin produced toxin after resisting the effect of light for two and one-half months and of drying on paper for twenty-two days.

In symbiosis with a yeast, it can be cultivated aerobically on agar slants. Grown with *B. coli* and a yeast in milk, it produces death when ingested by a rabbit. Grown with *M. aureus* in extract broth at 38-39 C., under aerobic conditions it likewise produced death when ingested by a rabbit.

In numerous instances the toxin produced death in the rabbit without any preliminary symptoms being observed. In one case in which the toxin was produced in +3 broth, death apparently was associated with infarct of the lung and embolus.

The nonpathogenicity of this strain is not definitely proved. In the intestines of the rabbit the organism apparently fails to grow, and injected subcutaneously the spores produce no symptoms. Yet the fact that it grows at body temperature in the presence of *M. aureus* renders it possible that in mixed infection in the throat or protected from the influence of the body tissues in some cavity of the teeth it may produce its toxin.

In the writer's experience few symptoms are to be observed in the rabbit when the toxin given is formed under aerobic conditions. Under these circumstances no definite paralysis of the limbs was observed, although the organism produced this symptom when grown anaerobically.

The postmortem findings of Ophüls and Dickson indicate that certain strains of *B. botulinus* may produce thrombosis and hemorrhage in the meninges and central nervous system.

These facts would seem to indicate that the toxin produced by the Nevin Strain might cause sudden death by embolus or hemorrhage in the brain, accompanied by but few preliminary symptoms of a minor character.

The culture is capable of killing donkeys when given on feed in doses as low as 0.2 c.c.

What is known of the etiology, symptomatology, and pathology of forage poisoning indicates that the disease may be caused by a toxin similar to that produced by *B. botulinus*.

No definite conclusions can be drawn from the few experiments performed, but in view of the facts mentioned it seems probable that some cases of forage poisoning may be due to certain strains of *Bacillus botulinus*.[6]

Finally, it should be observed that this organism presents manifold possibilities for use as a poison both for horses and men. The toxin of the variety of *B. botulinus* employed certainly possesses the power of killing horses in very minute doses when given in the feed.

In regard to the toxicity of the organism for man, it would seem at first glance that the symptoms produced would be those of botulism. For the organism was isolated by Nevin from cheese that had produced numerous cases of a disease diagnosed postmortem as botulism, and the symptoms of this disease are well known and should be capable of diagnosis.

But there always remains the possibility of the organism growing in the body and producing its toxin slowly and under altered conditions, thereby causing a different syndrome from that seen when the toxin is ingested.

It is noteworthy that the most marked, constant lesion produced in the rabbit, horse and donkey was a congestion of the lungs, *when the culture was ingested by the animal*. In the first preliminary experiment on the rabbit, and in one other in which the culture was injected subcutaneously, this lesion was not noticed, nor is it apparent in Nevin's[4] work in which injections were used throughout. Apparently then, the organism is capable of producing various lesions. Certainly the pathologic findings in rabbits which had ingested cultures grown in symbiosis with a yeast under aerobic conditions would not lead one to make the diagnosis of botulism if these lesions had been found in man. Again, the dosage would probably affect the symptoms. In large doses sudden death might be expected.

If placed in the water supply the organism might find conditions favorable for the production of a small amount of toxin, and since disinfectants have little effect on spore bearers, it is probable that treatment of such water by the hypochlorites would fail to kill the organism.

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6. Since this statement was written the work of L. H. Pammel (Forage Poisoning Due to Bacillus Botulinus, Am. J. Vet. M. **13**: No. 3, March, 1918) has strengthened the supposition.

THE MECHANISM OF THE PROTECTIVE ACTION OF CARBOHYDRATE DIET IN PHOSPHORUS AND CHLOROFORM POISONING *

J. P. SIMONDS, M.D.
CHICAGO

In a series of experiments reported elsewhere[1] it was shown that the esterase content of the livers of dogs poisoned with phosphorus or with chloroform was not materially less than that of the livers of normal dogs, and that the feeding of liberal amounts of sugar causes an increase in the amount of this ferment in both normal and phosphorus poisoned animals. In another series of experiments,[2] using the same extracts as in those just mentioned, it was found that phosphorus poisoning causes an appreciable reduction in the ereptic power of the liver, that the feeding of sugar does not increase the ereptase content of the livers of normal dogs, but that it does prevent the diminution of this ferment when fed to animals which have been poisoned with phosphorus. These findings have led to the formulation of certain ideas as to the mechanism of phosphorus and chloroform poisoning, and of the detoxicating, antitoxic or sparing action of carbohydrate diet in poisoning with these substances. These conceptions are presented in this article.

The changes in the liver in phosphorus and chloroform poisoning, in acute yellow atrophy and in eclampsia, have much in common. In phosphorus poisoning the liver is large and fatty, the fatty change beginning in the peripheral part of the hepatic lobule, actual necrosis of the liver cells occurring *relatively* late. In acute yellow atrophy, the pathologic process begins in the central zone of the lobule as a fatty degeneration, proceeds rather rapidly to necrosis, and may ultimately involve the entire lobule. In chloroform poisoning the lesion also starts centrally as a fatty degeneration followed by early necrosis. In eclampsia, the fatty degeneration and early necrosis begin in the periphery of the lobule. All four of these conditions, therefore, have in common fatty degeneration and necrosis of the liver cells, the differences being chiefly in the location of the lesion in the lobule, the amount of visible fat present and the rate at which necrosis occurs.

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* From the Laboratory of Pathology, Northwestern University Medical School.

1. Simonds, J. P.: J. Exper. M. **28**:663, 1918.
2. Simonds, J. P.: J. Exper M **28**:673, 1918

Of the four conditions mentioned, phosphorus poisoning shows the largest amount of visible fat. The intracellular droplets are relatively large so that the liver has the microscopic appearance of the so-called fatty infiltration. The work of Taylor,[3] Rosenfeld[4] and others has indicated that in phosphorus poisoning there occurs a transportation of fat to the liver from the fat depots of the body. But an additional change also takes place in the intracellular lipoids. Taylor[3] found that if the fat was extracted from livers and the residue digested with pepsin and trypsin, a greater amount of fat could be extracted from the digests of normal than of phosphorus poisoned livers; that is, practically all the fat in the phosphorus poisoned livers was in a free state. This indicates that a considerable part of the fat in the liver exists normally in an invisible, unextractable combination which is broken up by phosphorus poisoning. In other words, in this pathologic condition there is both fatty infiltration and fatty degeneration. In chloroform poisoning and in acute yellow atrophy the changes in the liver are more of the nature of a fatty degeneration without so much infiltration with transported fat. The cells which are affected undergo a rather rapid necrosis. Rosenfeld[4] observed that fat may become visible as fine droplets in dead or dying cells in the process of autolysis. For the taking up and storage of fat transported to the cells a certain amount of vital activity, as indicated by the presence of the nucleus, is essential (Dietrich[5]). In phosphorus poisoning the nucleus does not disappear so early and the cell retains its vitality for a sufficient time to permit the synthesis of fat from transported materials, and its accumulation within the cells in large droplets. Oppel[6] even considers that the appearance of fat in the liver cells in phosphorus poisoning is not due to degeneration but to a stimulation of the cells to an increased endogenous formation of fat from exogenous materials.

A further important change in the cells of the liver in phosphorus and chloroform poisoning is the rapid disappearance of glycogen. The glycogen of the cells of the body may, as pointed out by Gierke,[7] either form a more or less integral part of the cytoplasm, as in epithelium and cartilage, cells at some distance from the blood supply, and its amount is not readily affected by extracellular influences; or the glycogen may be the result of a process of storage, as in the cells of the liver and skeletal muscles. The quantity of storage glycogen is

3. Taylor, A. E.: J. M. Research **9**:59, 1903.
4. Rosenfeld, G.: Berl. klin. Wchnschr. **41**:587 and 617, 1904.
5. Dietrich, A.: Arb. a. d. path. Inst. Tübingen **5**: No. 1, 1904.
6. Oppel, A.: Arch. Entwickelungsmech. **30**: Part 1, 304, 1910.
7. Gierke, E.: Ergebn. d. spez. path. Morph. u. Physiol. d. Sinnesorg. (Lubarsch and Oestertag) **2**: Part 2, 871, 1907.

easily altered by external factors, either increasing or decreasing, according to the nature of the influence at work. It may entirely disappear from the liver in starvation, in infections and after various types of poisonings. Doyon[8] found decrease of glycogen of the liver after poisoning with abrin, a condition which Flexner[9] showed was associated with necrotic changes in the liver. Andrea[10] observed a similar reduction after injections of hemolytic serums which, as Pearce[11] and Karsner and Aub[12] have shown, are followed by localized necroses in the liver. Saikowski,[13] Rosenbaum,[14] Athanasiu,[15] Rettig,[16] and others have observed a rapid and complete disappearance of glycogen from the liver in phosphorus poisoning. Rettig also found that the liver lost its stored glycogen in chloroform poisoning.

Reports in the literature of studies of the enzymes in the conditions under discussion are not numerous. Whipple,[17] Jobling, Eggstein and Peterson,[18] Quinan,[19] Sagal[20] and Simonds[1] have observed an increase in the serum esterase in phosphorus poisoning. Whipple,[17] Quinan[19] and Simonds[1] noted a similar increase in chloroform poisoning. Sagal[20] and Whipple[17] record cases of puerperal eclampsia in which the serum esterase was augmented. According to Whipple,[17] increase of the esterase of the serum indicates a destructive lesion of the liver.

The results of studies of the esterase content of the liver itself in these pathologic processes are strikingly conflicting. Jobling, Eggstein and Peterson[18] and Quinan[19] report a reduction of the esterolytic power of the liver in phosphorus poisoning. Winternitz and Meloy[21] say that when microscopic fat is present in the liver its esterase content is reduced. Loevenhart,[22] Duccheschi and Almagia,[23] Saxl[24] and Simonds[1] found little or no change in the ester splitting activity of the livers of animals poisoned with phosphorus.

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8. Doyon: Compt. rend. Soc. de biol. **67**:30, 1909.
9. Flexner, S.: Johns Hopkins Hosp. Rep. **6**:259, 1897.
10. Andrea: Arch. internat. de pharmacod. **14**:177, 1905.
11. Pearce, R. M.: J. M. Research **12**:329, 1904.
12. Karsner, H. T., and Aub, J. C.: J. M. Research **28**:377, 1913.
13. Saikowsky: Virchow's Arch. f. path. Anat. **34**:73, 1865.
14. Rosenbaum, F.: Arch. f. exper. Path. u. Pharmakol. **15**:450, 1882.
15. Athanasiu, J.: Arch. f. d. ges. Physiol. **74**:511, 1899.
16. Rettig, H.: Arch. f. exp. Path. u. Pharmakol. **76**:345, 1914.
17. Whipple, G. H.: Bull. Johns Hopkins Hosp. **24**:357, 1913.
18. Jobling, J. W.: Eggstein, A. A., and Peterson, W.: J. Exper. M. **22**:707, 1915.
19. Quinan, C.: J. M. Research **33**:73, 1915.
20. Sagal, Z.: J. M. Research **34**:231, 1916.
21. Winternitz, M. C., and Meloy, C. R.: J. M. Research **22**:107, 1910.
22. Loevenhart, A. S.: Am. J. Physiol. **6**:331, 1902.
23. Duccheschi, V., and Almagia, M.: Arch. ital. de biol. **39**:29, 1903.
24. Saxl, P.: Biochem. Ztschr. **12**:343, 1908.

The literature on intracellular ereptase in these conditions is still more meager and contradictory. Bergell and Lewin[25] report that the ereptic ferment of the liver is destroyed in phosphorus poisoning. Abderhalden and Schittenhelm[26] on the other hand, claim that the ereptase content of the livers of phosphorus poisoned dogs is as great if not greater than that of normal dogs. In my own experiments[2] there was found an appreciable reduction of the power of liver extracts of phosphorus poisoned dogs to split peptone into amino-acids.

Endogenous protein metabolism in all four of the conditions mentioned is increased. Bauer[27] found protein metabolism in phosphorus poisoning 295 per cent. greater than normal. Very nearly identical figures were obtained by Lo Monaco[28] and by Ray, McDermott and Lusk.[29] Von Noorden[30] found a pathologic increase in protein metabolism in a woman poisoned with phosphorus. Other important studies based on the partition of urinary nitrogen and all indicating increased protein metabolism have been made by Badt,[31] Münzer,[32] Pfaundler,[33] Marshall and Rowntree,[34] Welsch,[35] Lusk,[36] Riess[37] and Porgess and Pribraum[38] in phosphorus poisoning; by Howland and Richards,[39] and Lindsay[40] in chloroform poisoning; and by Richter,[41] Münzer[32] and Riess[37] in acute yellow atrophy. In general, it may be said that urea excretion is decreased and ammonia excretion is increased. The increase of urinary ammonia appears to be partly the result of excessive formation of organic acids due to imperfect oxidation. For Münzer[32] was able to reduce the urinary ammonia in a phosphorus poisoned animal to almost normal by the administration of sodium bicarbonate. Amino-acids, especially leucin and tyrosin, are occasionally found in the urine in phosphorus poisoning, but are usually present in acute yellow atrophy, according to the findings of Ries.[37]

25. Bergell, and Lewin, C.: Ztschr. f. exper. Path. u. Therap. **3**: 1907.
26. Abderhalden, E., and Schittenhelm, A.: Ztschr. f. physiol. Chem. **49**:40, 1907.
27. Bauer: Ztschr. f. Biol. **14**:527, 1878.
28. Lo Monaco: Cited by Ray, McDermott and Lusk, Footnote 29.
29. Ray, W. E., McDermott, T. S., and Lusk, Graham: Am. J. Physiol. **3**:139, 1900.
30. Von Noorden, C.: Metabolism and Practical Medicine, London **2**:10, 1907.
31. Badt: Diss., Berlin, 1891; Abstracted in Chem. Centralbl. **2**:265, 1891.
32. Münzer, E.: Deutsch. Arch. f. klin. Med. **52**:199, 1895.
33. Pfaundler, M.: Ztschr. physiol. Chem. **30**:75, 1900.
34. Marshall, E. K., Jr., and Rowntree, L. G.: J. Exper. M. **22**:333, 1915.
35. Welsch, H.: Arch. internat. de pharmacod. **14**:211, 1905.
36. Lusk, Graham: Am. J. Physiol. **19**:461, 1907.
37. Riess, L.: Berl. klin. Wchnschr., **42**: 1895. Ewald Fest Number, p. 54.
38. Porges, O., and Pribram, E.: Arch. f. exper. Path. u. Pharmakol. **59**:20, 1908.
39. Howland, J., and Richards, A. N.: J. Exper. M. **11**:344, 1909.
40. Lindsay, D. E.: Biochem. J. **5**:407, 1910-1911.
41. Richter: Berl. klin. Wchnschr., **43**:453, 1896.

Howland and Richards[39] and Lindsay[40] found abnormalities in the creatinin and creatin of the urine.

That the greater part of this tissue disintegration occurs in the liver is evidenced by numerous analyses. Slowtzoff,[42] in phosphorus poisoning, found an actual decrease in the weight of the liver in proportion to body weight from 3.6 per cent. (normal) to 2.5 per cent; the protein of the liver was reduced from 18.7 per cent. (normal) to 12.86 per cent., while the fat content was nearly double that of normal livers. Wakeman's[43] analyses showed that the total nitrogen of normal livers was 11.41 parts in 100 parts of dried liver substance, while in phosphorus poisoned livers it was only 7.34 parts, a loss of 35.67 per cent. The phosphorus poisoned livers also contained 37.1 per cent. less hexone bases than did normal livers. Similar but less marked changes were found by Wakeman in the livers of animals poisoned with chloroform. Porges and Pribram[38] also observed a reduction in the hexone bases of the liver in phosphorus poisoning. Wells[44] made chemical analyses of the liver in acute yellow atrophy and isolated and identified eight different amino-acids; the amount of soluble nonprotein nitrogen was very large; and there were small quantities of free proteoses and peptones and of xanthin and hypoxanthin.

That the liver is the source of much of the intoxication in phosphorus and chloroform poisoning and in acute yellow atrophy is evidenced by a number of facts. Thus Fischler and Bardach[45] found that dogs with Eck fistulas are more resistant to phosphorus poisoning than are normal dogs. Opie and Alford[46] showed that a carbohydrate diet, which causes an abundant deposit of glycogen in the liver, protects strongly against poisoning with phosphorus and chloroform. Graham[47] demonstrated that the resistance of dogs to the delayed toxic effect of chloroform is proportional to the amount of glycogen in the liver. Rettig[16] made a similar observation in connection with phosphorus poisoning. In a previous article[1] the astonishing differences between the clinical manifestations of phosphorus poisoning in dogs with and without the feeding of sugar was emphasized. It was further shown that the livers of those animals which had received sugar contained glycogen, and that the degenerative changes

42. Slowtzoff, B.: Biochem. Ztschr. **31**:227, 1911.
43. Wakeman, A. J.: J. Exper. M. **7**:292, 1905.
44. Wells, H. G.: J. Exper. M. **9**:627, 1907.
45. Fischler and Bardach: Ztschr. f. physiol. Chem. **78**:435, 1912.
46. Opie, E. L., and Alford, L. B.: J. A. M. A. **62**:295, 1914; J. Exper. M. **21**: pp. 1 and 21, 1915.
47 Graham, E. A.: J. Exper. M. **21**:185, 1915.

were less marked than in those to which sugar had not been fed and whose livers contained little or no glycogen.

The source of the toxic material for which the liver appears to be responsible in these conditions is not definitely known. Van Slyke and Losee[48] concluded that the responsibility for the toxemias of pregnancy cannot be left with the amino-acids nor with the intermediate products of protein metabolism. A similar conclusion was reached by Howland and Richards[39] who decided that death from delayed chloroform poisoning was probably due to the presence of toxic substances of an unknown nature which owe their presence to excessive formation by abnormal processes or to the failure on the part of the organism to neutralize in the normal manner toxic substances normally formed. Schryver[49] found that the products of the incipient stage of autolysis of the liver were highly toxic.

We may now summarize the conditions found in phosphorus poisoning. There is an increased catabolism of tissue protein as evidenced by changes in the distribution of urinary nitrogen; by the occasional excretion in the urine of amino-acids, especially leucin and tyrosin; and by the frequent presence in the urine of abnormal amounts of creatin and creatinin. That the liver is the chief sufferer among the organs in this increased tissue destruction is shown by the absolute loss of weight, and the decrease of protein and protein nitrogen in the organ, and by the presence in the liver of various products of protein decomposition. There is, further, a marked increase of the fat of the liver, due in part to the unveiling of hidden or bound fat, and in part to the transport to the hepatic cells of fat which they are unable to burn. There is in addition a rapid and complete disappearance of glycogen from the liver. Finally, there is a diminution of intracellular ereptase with little or no change in the intracellular esterase.

Studies in the chemical and enzymatic changes in the liver, and of metabolism, in chloroform poisoning, acute yellow atrophy and eclampsia are not so complete as for phosphorus poisoning. But from the reports recorded in the literature, many of which have been cited, there is reason to believe that the conditions in these pathologic states are not greatly unlike those summarized in the foregoing for phosphorus poisoning. The differences are probably more quantitative than qualitative, and depend on differences in the rate at which actual necrosis of the injured liver cells takes place.

How can we correlate the foregoing conditions with the marked protective, detoxicating or antitoxic effect of a carbohydrate diet in

48. Van Slyke, D. D., and Losee: Quoted by Van Slyke, D. D., Arch. Int. Med. **19**:56, 1917.

49. Schryver, S. B.: Biochem. J. **1**:123, 1906.

poisoning with phosphorus and chloroform as has been demonstrated by Opie and Alford,[46] Graham,[47] Rettig,[16] and Simonds?[21] That the presence of a usable sugar may profoundly affect cellular metabolism was shown many years ago when Theobald Smith[50] demonstrated that the presence of an adequate supply of fermentable carbohydrate greatly reduced or even entirely inhibited the production of toxins by *B. diphtheriae* and *B. tetani*. Kendall,[51] Kendall and Farmer,[52] and Kendall, Day and Walker[53] have shown for a large number of different bacteria that when a fermentable carbohydrate is available, the protein of the medium is only slightly attacked, while in sugar-free mediums there is a very much greater decomposition of the protein by the bacteria. Kendall and Walker[54] found that in the presence of a fermentable sugar, *B. proteus* does not produce the extracellular enzyme which liquefies gelatin; and further that if a small amount of dextrose, up to 0.3 per cent., is present, the formation of the enzyme and the liquefying of the gelatin only begins after all of the sugar has been used up. Simonds[55] found that a culture of *B. typhosus* grown on dextrose medium showed antigenic properties and serologic reactions somewhat different from those of the same strain accustomed to growing on sugar-free mediums.

The presence of a readily available sugar also modifies the metabolism of animal cells. Indeed, the presence of a carbohydrate appears to be essential to intracellular metabolism. Landergren[56] studied protein metabolism on a diet as nearly protein-free as possible, but with sufficient calories supplied by carbohydrates. During fasting there is much breaking down of body protein. If, now, the requisite number of calories are furnished by a diet of nitrogen-free carbohydrate, endogenous metabolism is promptly decreased to an irreduceable minimum. Landergren believes that there is a permanent need for carbohydrates in the body, and if these are not supplied in the diet, more protein is broken down in order to form the necessary sugar from the decomposition products of the protein. Lüthje[57] was led by his experiments to the conclusion that the presence of carbohydrate is essential for the synthesis of protein in the body from the products

50. Smith, Theobald: Tr. Assn. Am. Phys. **11**:37, 1896; J. Exper. M. **4**:373, 1899.
51. Kendall, A. I.: J. M. Research **25**:117, 1911.
52. Kendall, A. I., and Farmer, C. J.: J. Biol. Chem. **12**:13, 19, 215, 219 and 465; **13**:63, 1912.
53. Kendall, A. I., Day, A. A., and Walker, A. W.: J. Am. Chem. Soc. **35**: 1201, 1913, and **36**:1937, 1914.
54. Kendall, A. I., and Walker, A. W.: J. Infect. Dis. **17**:442, 1915.
55. Simonds, J. P.: J. Infect. Dis. **17**:500, 1915.
56. Landergren, E.: Skand. Arch. f. Physiol. **14**:112, 1903.
57. Lüthje, H.: Arch. f. d. ges. Physiol. **113**:547, 1906.

of digestion of protein. Cathcart[58] also puts forward the hypothesis that the carbohydrates are absolutely essential for endocellular synthetic processes in connection with protein metabolism. Rolly[59] rendered the livers of rabbits glycogen-free by an initial dose of strychnin followed by starvation. Glycogen reappeared in the livers in very small amounts in spite of the starvation, and at the height of increased protein metabolism which accompanied the starvation. Rolly believed that this glycogen was formed from the decomposition products of the protein and that its formation is due to an effort on the part of the organism always to possess a certain amount of glycogen.

Inasmuch, therefore, as the presence of carbohydrate is of very great importance, and even appears to be essential to normal intracellular metabolism, it is all the more important that it be supplied in those conditions in which there is increased protein metabolism, as in phosphorus and chloroform poisoning, acute yellow atrophy of the liver and probably eclampsia. Indeed, in these conditions it is possible that a sort of vicious circle may be produced. Lusk[60] has shown that in phosphorus poisoning there is no sugar left in the body; and Aronsohn[61] has pointed out that impoverishment of the body cells in carbohydrate and fat results in increased protein metabolism. The effect of phlorizin is to sweep the body clean of sugar. Reilly, Nolan and Lusk[62] found that after subcutaneous injections of this drug protein metabolism may be increased 560 per cent.

What has just been said applies to the relation of carbohydrates to general metabolism. But it is the liver that shows the most serious lesions in at least three of the four conditions mentioned. And it is the liver that is one of the most important storehouses of glycogen. We are, therefore, also concerned with the local protective action of carbohydrate on the liver itself. Mathews[63] has given reasons which make it probable that glycogen is stored in the liver primarily for the benefit of the liver itself, and that it is only incidentally liberated as glucose when it is demanded by the other tissues of the body. That the presence of an abundant supply of glycogen in the liver does exert a local protective action is evidenced (1) by the effect on intracellular enzymes, as shown by Simonds (Footnotes 1 and 2); and (2) by the entire absence or greater mildness of the lesions in the livers of sugar fed, phosphorus poisoned dogs, as compared with nonsugar fed, phos-

58. Cathcart, E. P.: Jour. Physiol. **39**:311, 1909-1910.
59. Rolly, Fr.: Deutsch. Arch. f. klin. Med. **83**:107, 1905.
60. Lusk, Graham: Am. Jour. Physiol. **1**:5, 1898.
61. Aronsohn, Ed.: Ztschr. f. klin. Med. **61**:153, 1907.
62. Reilly, F. H.: Nolan, F. W., and Lusk, Graham: Am. J. Physiol. **1**:395, 1898.
63. Mathews, A. P.: Physiological Chemistry, New York, p. 786, 1915.

phorus poisoned dogs as shown by Opie and Alford,[46] Rettig[16] and Simonds.[1]

In its mechanism this local protective action may involve several factors. In the first place, the metabolism of the liver itself is increased. The presence of a supply of carbohydrate ready at hand spares the protein of the hepatic cells. Whipple and Hooper[64] have shown that the feeding of carbohydrates stimulates the excretion of bile in bile-fistula dogs. It is not impossible that the same applies to other functions of the liver cells and that the presence of an adequate supply of sugar will stimulate these cells to increased function and will render them more resistant to the injurious action of the poison.

It is Wells' view[65] that in chloroform and phosphorus poisoning there is an interference with the oxidative processes in the liver, and that this accounts for the accumulation of fat in this organ in these conditions. Lusk[66] failed to find any reduction in oxidations in the body in phosphorus poisoning so far as general metabolism is concerned. Duccheschi and Almagia[67] found no reduction in the oxidizing enzyme aldehydase in phosphorus poisoned livers. Joannovics and Pick,[68] however, think that the interference by chloroform with oxidation in the liver is probably a selective one, and that one or more, but not all, of the manifold vitally important oxidative processes of the liver are put out of commission by the narcotic. Slowtzoff[42] claims to have found less peroxidase in the livers of phosphorus poisoned animals.

Reduced oxidation in the body in phosphorus poisoning is further indicated by the presence of organic acids, especially lactic acid, in the urine as observed by Araki,[69] Hauser,[70] Riess,[37] and Mandel and Lusk.[71] In a study of the respiratory exchange in phosphorus poisoning Scheider[72] found a 26 per cent. decrease in the excretion of carbon dioxid; while Welsch[35] found a 11 to 20 per cent. decrease.

The observations just cited indicate that there is a condition of reduced oxidation in the body in phosphorus and chloroform poisoning. But this does not necessarily mean that this is due to such a reduction in the ability of the body to carry on its oxidative processes as has been supposed. The reduced oxidation may be due in part to

64. Whipple, G. H., and Hooper, C. W.: Am. J. Physiol. **40**:349, 1916.
65. Wells, H. G.: J. A. M. A. **46**:341, 1906.
66. Lusk, Graham: Science of Nutrition, Philadelphia, 1909.
67. Duccheschi, V., and Almagia, M.: Arch. di farmacol. sperim. **2**:1, 1903.
68. Joannovics, G., and Pick, E. P.: Arch. f. d. ges. Physiol. **140**:327, 1911.
69. Araki, T.: Ztschr. f. physiol. Chem. **17**: 1893.
70. Hauser, A.: Arch. f. exper. Path. u. Pharmakol. **36**:165, 1895.
71. Mandel, A. R., and Lusk, G.: Am. J. Physiol. **16**:129, 1906.
72. Scheider: Dissertation, Würzburg, 1895. Cited by Loewi: v. Noorden's Metabolism in Practical Medicine, London, 1907, p. 1108.

the depletion of the supply of readily oxidizable material. That the glycogen in the liver at the time of poisoning is at least partially oxidized is evident from the fact, noted by Laub,[73] Walko,[74] Neubauer,[75] and others, that in spite of the very rapid glycogenolysis, there is only very exceptionally a hyperglycemia or glycosuria. Neubauer and Porges[76] even found a hypoglycemia in phosphorus poisoning. Neubauer[76] distinguishes between two groups of conditions in which glycogen disappears from the liver. In one group the glycogen is used up because of increased need, as in starvation, muscular work, fever, etc. In this group, to which phosphorus poisoning belongs, hyperglycemia and glycosuria do not occur. In the second group, the glycogenolysis is the result of nervous stimuli, as after stimulation of the splanchnics, injection of epinephrin, and possibly after poisoning by phlorizin. In these cases, hyperglycemia and glycosuria do occur.

It is frequently stated that the deposit of fat and of glycogen in the liver results from the same general cause, namely, reduced oxidation. Rosenberg[77] and Rosenfeld,[78] however, found evidence of antagonism between glycogen and fat. Mathews[79] takes the view that glycogen is stored in the liver at a time when the portal blood is relatively rich in oxygen; that is, during digestion, and that it tends to disappear when, during the fasting period, the oxygen content of the portal blood is very low. This view is supported by the observation of Araki[80] that glycogen of the liver is reduced in carbon dioxid poisoning. There is reason to believe that the primary object of the supply of stored glycogen is to enable the liver to function properly in the presence of very little oxygen (Mathews). The work of Embden and Wirth[81] shows that the liver can burn its own glycogen more readily than it can oxidize glucose or a number of other organic substances supplied to it. Rettig[16] is of the opinion that the primary effect of phosphorus is not a direct toxic injury to the cells, but that it is the result of the absence of easily oxidizable carbohydrate.

Mathews[82] has advanced the hypothesis that intracellular respiration is a splitting off of water from the cytoplasm and its dissociation

73. Laub, M.: Wien. klin. Wchnschr. **10**:27, 1898.
74. Walko, K.: Ztschr. f. Heilk. **22**:8, 1901.
75. Neubauer, E.: Arch. f. exper. Path. u. Pharmakol. **61**:387, 1909.
76. Neubauer, E., and Porges, O.: Biochem. Ztschr. **32**:290, 1910.
77. Rosenberg, O.: Ziegler's Beitr. z. path. Anat. u. z. allg. Path. **49**:284, 1910.
78. Rosenfeld, G.: Allg. Med. Central-Ztg., 1897; Ergebn. d. Physiol. **1**:650, 1901.
79. Mathews, A. P.: Physiological Chemistry, New York, 1907, p. 786.
80. Araki, T.: Ztschr. f. physiol. Chem. **19**:422, 1894.
81. Embden, G., and Wirth, J.: Biochem. Ztschr. **37**:1, 1910.
82. Mathews, A. P.: Biolog. Bull. **8**:331, 1905.

with the liberation of nascent hydrogen and oxygen. This nascent oxygen accomplishes the oxidative processes within the cell. The hydrogen is disposed of by combining either with atmospheric oxygen, if this is present (aerobic respiration), or, if this is absent, with some other available easily oxidizable substance (anaerobic respiration). According to this hypothesis, the atmospheric oxygen absorbed in the lungs and circulating in the blood, merely acts as a depolarizer to take care of the nascent hydrogen. Any other substance which can easily combine with the hydrogen will serve the purpose of the atmospheric oxygen and permit respiration to go on in the absence of air. Mathews[83] further points out that while carbohydrates are readily oxidized and thus act as reducing agents, they are also reduced without difficulty and may act as oxidizing agents. By means of this property of being easily reduced, they play an important part in anaerobic respiration. Packard[84] has shown that those carbohydrates which can be absorbed when injected into the peritoneum of the common minnow (*Fundulus heteroclitis*) greatly increase its resistance to lack of oxygen. It has been shown many times that perfused organs will retain their vitality for a much longer time if the perfusion liquid contains glucose.[85] It is not an unreasonable supposition, therefore, that an adequate supply of readily oxidizable glycogen will help to maintain the oxidative activity of the liver in the presence of interference within intracellular oxidations due to phosphorus and chloroform poisoning.

The contents of the hepatic and other cells of the body are colloidal in nature. Some of the degenerative changes occurring in cells may be explained on the basis of reversible colloids. Certain substances, among them glycogen and the sugars, have a protective action and tend to prevent the "breaking" of emulsions and the precipitation of colloids. Fischer[86] lays great emphasis on this protective action of glycogen in the liver in phosphorus poisoning. The local reduced oxidation results in the accumulation of acids in the liver. These cause the cells to take up water which "breaks" the emulsion and droplets of fat appear. The presence of abundant glycogen, however, stabilizes the emulsion and prevents fatty degeneration, which, according to Fischer, is a purely physicochemical phenomenon governed by physicochemical laws.

Not only do certain colloids protect others from precipitation, but the permeability of colloids can be modified by various means.

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83. Mathews, A. P.: Physiological Chemistry, New York, 1915, p. 41.
84. Packard, W. H.: Am. J. Physiol. **18**:164, 1907, and **21**:310, 1908.
85. Locke, F. S.: J. Physiol. **31**:13, 1904, and Centralbl. f. Physiol. **14**:670, 1901.
86. Fischer, M. H.: Fats and Fatty Degeneration, New York, 1917, p. 74.

Bechhold and Ziegler[87] found that glucose retarded the diffusion of some substances into gels. It is not fully known in just what condition phosphorus reaches the liver to exert its toxic effect. Plavac[88] has studied this point particularly, and concluded that the phosphorus enters into some combination, and that this compound acts as a poison instead of the phosphorus being resorbed and circulating and acting as such. In whatever form the phosphorus finally reaches the liver, it appears probable that it will find the cells less permeable if they are amply supplied with glycogen.

In a previous paper[1] it was shown that the feeding of sugar increases the esterase content of the livers of both normal and phosphorus poisoned animals. In those animals poisoned with phosphorus and fed with sugar in large amounts the livers contained much glycogen, and showed less fatty change than the nonsugar fed control animals. It was suggested that the relatively small amount of fat in the livers of the sugar fed animals was in some way closely related to the marked increase of the esterase.

The metabolism of fats is not yet fully understood, but it is probable that the intracellular lipases synthesize fat in the cells from materials brought to them by the circulating fluids. Hanriot[89] observed such synthesis *in vitro* in the case of butyric acid and glycerol which formed monobutyrin under the influence of lipase; and Pottevin[90] succeeded in synthesizing triolein. Bradley,[91] it is true, studied the lipase content of different tissues in relation to the amount of fat which they contained and found no parallelism between the two. He concluded from this that lipase does not synthesize fat. Bayliss,[92] however, has presented reasons why this observation of Bradley's does not negative the hypothesis of the synthesis of fat by lipase.

Wells[65] is of the opinion that in the presence of the inhibition of oxidation in the liver cells in phosphorus poisoning the lipase synthesizes fat, and he thus accounts for the accumulation of fat in this organ in phosphorus and chloroform poisoning. The finding of the writer[1] that there is no reduction in the esterolytic activity of the liver in phosphorus poisoning harmonizes with this view. On the other hand, the observation recorded in the same paper,[1] that the feeding of sugar increases the hepatic esterase in phosphorus poisoned dogs and that the livers of these sugar fed animals showed less fatty change than did the controls, seems, at first sight, to be contradictory. Rosen-

87. Bechhold, H., and Ziegler, J.: Ztschr. f. physikal. Chem. **56**:105, 1906.
88. Plavac, V.: Arch. f. ges. Physiol. **104**:1, 1904.
89. Hanriot, M.: Compt. rend. Soc. de biol. **132**:212, 1901.
90. Pottevin, H.: Compt. rend. Soc. de biol. **136**:767, 1903.
91. Bradley, H. C.: Jour. Biol. Chem. **13**:407, 1913.
92. Bayliss, W. M.: Nature of Enzyme Action, New York, 1914, p. 53.

feld[93] claims that a deficiency of carbohydrate in the diet is the essential condition to the deposit of fat in the body. Rosenberg[77] and also Rosenfeld[78] noted "a certain antagonism" between fat and glycogen. Rosenberg[77] observed that in fatty degeneration the cells were poor in glycogen, and that if glycogen were supplied the fatty degeneration diminished or disappeared. Lusk[80] concluded that as a result of the depletion of the glycogen of the liver in phosphorus poisoning the "sugar hungry" cells attract fat in greater quantity than they can burn.

In the oxidation of fats in the body it is probable that they are first hydrolyzed by the intracellular lipases. With the "sugar hungry" cells attracting an augmented amount of the crude materials from which fat is synthesized, the equilibrium point for the activity of the lipase would be well toward the side of synthesis. The hydrolysis and subsequent oxidation of fats within the cells would therefore be interfered with. The presence of easily oxidizable glycogen in the liver cells reduces the demand for fat, so that the equilibrium point would not be disturbed by the attraction of an excessive supply of raw materials.

The changes occurring in the liver in phosphorus poisoning have been very generally charged to the process of autolysis. Thus Jacoby[94] found that autolysis was very much more rapid in the livers of phosphorus than in normal dogs. Wells[65] concluded that chloroform, phosphorus and possibly other poisons stop cell activity, leaving the autolytic enzymes free to digest the necrosed cells. Jackson and Pearce,[95] and Frank and Isaac,[96] however, call attention to certain differences in the chemical processes occurring in a liver undergoing rapid or immediate necrosis and those accompanying "slow degeneration, as in phosphorus poisoning." They believe that although investigation on the livers of phosphorus poisoned animals tends to show that the change is an autolysis in which amino-acids are split off from the protein molecule, it is not of the same type as that occurring in a completely necrotic cell. That there is some justification for this differentiation is evident from a comparison of the results of chemical analyses obtained by Jackson and Pearce[95] in liver necrosis due to injection of hemolytic immune serum, and by Wells[44] in acute yellow atrophy of the liver, with the results of chemical analyses by Wakeman[43] in phosphorus and chloroform poisoning, and by Wells[97] in chloroform poisoning.

93. Rosenfeld, G.: Berl. klin. Wchnschr. **43**:978, 1906.
94. Jacoby, M.: Ztschr. f. physiol. Chem. **30**:174, 1900.
95. Jackson, H. C., and Pearce, R. M.: J. Exper. M. **9**:520 and 554, 1907.
96. Frank, E., and Isaac, S.: Arch. f. exper. Path u. Pharmakol. **64**:274, 1910.
97. Wells, H. G.: Arch. Int. Med. **1**:589, 1908.

From the standpoint of the treatment of these conditions this is a most important distinction. For as Wells[65] suggests, if enough liver cells have been destroyed, hepatic insufficiency may cause death. The logical desideratum in treatment will therefore be to prevent the "slow degenerative process" from going on to actual necrosis.

It appears, furthermore, that the amount of glycogen in the liver in some way affects the rate of autolysis in that organ. The liver in phosphorus poisoning is free of glycogen, and as already mentioned, Jacoby[94] and others have shown that the livers of such animals undergo very rapid autolysis. Schryver[98] found that the autolytic processes were greatly accelerated in the livers of animals which had been given thyroid extract. Kuriyama[99] observed that the feeding of thyroid extract caused a rapid disappearance of glycogen from the liver. This may explain Wells'[100] failure to find any increase in autolysis when thyroid extract was added to autolyzing liver *in vitro*. Furthermore, Bradley[101] found that of the three types of muscle tissue, the striated form autolyzed most slowly, while heart and smooth muscle autolyzed from 50 to 100 per cent. more rapidly than did the skeletal muscle. In this connection Cremer's[102] analyses of the glycogen content of the different forms of muscle are of special significance. Cremer found 1.85 per cent. glycogen in skeletal muscle and 0.12 per cent. in heart muscle. Starvation also frees the liver of glycogen. Lane-Claypon and Schryver[103] found that not only was the latent period in autolysis much shorter in the livers of starved cats than in the livers of cats on the usual diet, but that the degradation of the protein was much more rapid and complete.

Mention has already been made of the increased protein metabolism in phosphorus and chloroform poisoning. In this connection it is significant that, as shown by the analyses of Bauer,[27] Münzer,[32] Marshall and Rowntree,[34] Howland and Richards[39] and others, the increased protein metabolism does not become manifest until the second or third day — that is, until the liver is free from glycogen. That this latent period is not due entirely to the slowness in absorption of the phosphorus is evident from the fact that it is the poison which causes the using up of the glycogen in the liver. Attention may here be called again to the similarity between the effect of diminished oxygen supply and phosphorus poisoning as pointed out by Bauer,[27]

98. Schryver, S. B.: J. Physiol. **32**:159, 1905.
99. Kuriyama: Am. J. Physiol. **43**:481, 1917.
100. Wells, H. G.: Am. J. Physiol. **11**:35, 1904.
101. Bradley, H. C.: Jour. Biol. Chem. **33**:11, 1918.
102. Cremer, A.: Ztschr. f. Biol. **24**:75.
103. Lane-Claypon, J. E., and Schryver, S. B.: J. Physiol. **31**:169, 1904.

Loewi,[104] Wells[65] and others. Loewi has tabulated the similarities in parallel columns. Terray[105] found that the increased nitrogen output does not occur immediately after the oxygen supply is reduced, but on the succeeding days.

In a previous paper[2] it was shown that the ereptic power of the liver was reduced in phosphorus poisoning and that the feeding of sugar prevented a decrease but did not cause any increase of this ferment. The function of the intracellular ereptase is not fully understood. However, since Vernon[106] found it present in the cells of all of the organs of the body, it appears probable that it is concerned in intracellular metabolism, most likely serving to synthesize amino-acids into peptones. Synthesis has not been satisfactorily demonstrated *in vitro* for any of the proteolytic ferments. Both Oppenheimer[107] and Cohnheim[108] question the assignment of a reversible reaction to the proteoclastic enzymes. But Bayliss[109] has made it clear that "although the direct evidence on the subject of protein synthesis is at present meager, the phenomena seen in trypsin digests are quite what would be expected if equilibrium in a reversible reaction be the explanation of what takes place. Such phenomena are (1) retardation due to accumulation of the products of the reaction, (2) recommencement of a reaction which had apparently come to an end, if the products be removed by dialysis, or other means, or if their concentration be reduced by dilution." The work of Folin[110] also supports the belief that the intracellular ereptase synthesizes protein from the amino-acids absorbed from the digestive tract.

Van Slyke and Meyer[111] have shown that amino-acids disappear very rapidly from the blood during digestion, being taken up and temporarily fixed by the tissues. Living cells do not normally contain demonstrable amounts of peptone. Hence the equilibrium point in the living cell is well on the side of the synthesis of amino-acids into peptone. Furthermore, the same cells contain tryptase and peptase,[112] so that any peptone synthesized by the ereptase would be further synthesized into protein by the associated intracellular enzymes. Furthermore, since the actual demand for nitrogen by the body is relatively small, the total amount of synthesis will not be great, the

104. Loewi, O.: v. Noorden's Metabolism and Practical Medicine, London, 1907, p. 1128.
105. Terray: Arch. f. d. ges. Physiol. **65**:397, 1897.
106. Vernon, M. H.: Jour. Physiol. **33**:81, 1905.
107. Oppenheimer, C.: Die Fermente und ihre Wirkungen, Leipzig, 1910, Ed. 3, p. 281, etc.
108. Cohnheim, O.: Enzymes, New York, 1912, p. 90.
109. Bayliss, W. M.: Nature of Enzyme Action, New York, 1914, p. 69.
110. Folin, O.: J. A. M. A. **63**:823, 1914.
111. Van Slyke, D. D., and Meyer, G. M.: J. Biol. Chem. **12**:399, 1912.
112. Vernon, M. H.: Intracellular Enzymes, London, 1908, and Dochez, A. R.: J. Exper. M. **12**:666, 1910.

major portion of the amino-acids temporarily stored being ultimately eliminated as urinary nitrogen. It is only when the stored amino-acids of the tissues are exhausted by starvation or by increased metabolism, as in phosphorus poisoning, that the equilibrium point is displaced to the side of intracellular proteolysis and peptolysis. There is in addition, in prolonged starvation and in phosphorus poisoning, a disappearance of the glycogen with removal of its inhibiting action on the autolytic ferments, which results in a still more vigorous breaking down of the protein of the body, partly for the purpose of furnishing carbohydrate for the "sugar hungry cells."

Hence, it would appear that under normal conditions the function of ereptase is to synthesize amino-acids into peptones. In phosphorus and chloroform poisoning, in acute yellow atrophy and perhaps in eclampsia, changes occur in the liver of the nature of autolysis, or a modified autolysis in the sense of Jackson and Pearce.[95] There are thus liberated substances on which ereptase exerts its disintegrative power. This explains the not infrequent finding of amino-acids, especially leucin and tyrosin, in the urine in phosphorus and chloroform poisoning, and their almost constant presence in acute yellow atrophy (Riess[37]).

Schryver,[113] who observed that the livers of starved animals autolyzed more promptly and more rapidly than did the livers of fed animals, has advanced the interesting idea that the liberation and activation of the autolyzing enzymes in the cells during life is a normal mechanism whose function it is to protect the organism against starvation. These enzymes come into action only when the energy needs of metabolism are not satisfied by the foodstuff ingested. Cohnheim[114] has pointed out certain alleged differences between the products of digestion by autolytic enzyme and by erepsin. It would seem that the autolytic process can be more easily explained as the result of the interaction of several enzymes. Ereptase is only one of the ferments concerned. Ereptase is unable to affect unchanged protein. In a considerable series of unpublished experiments it was found that extracts of livers which split peptone into amino-acids with ease produced no measurable proteolysis of horse or dog serum either with or without a preliminary treatment of the serum with chloroform according to the method of Jobling and Peterson.[115] Hence it would seem that in phosphorus and chloroform poisoning and probably in acute yellow atrophy and eclampsia, the normal activity of the intracellular enzymes, including ereptase, is reversed.

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113. Schryver, S. B.: Biochem. J. **1**:123, 1906.
114. Cohnheim, O.: Ztschr. f. physiol. Chem. **35**:134, 1902.
115. Jobling, J. W., and Peterson, W.: J. Exper. M. **19**:459, 1914.

SUMMARY AND CONCLUSIONS

In phosphorus poisoning there is a rapid disappearance of glycogen from the liver. Beginning about the time of the disappearance of the glycogen, there occur fatty degeneration and infiltration of the liver, increased protein metabolism, and decreased oxidation. In addition there is a reduction of the intracellular ereptase with little or no change in the esterase content of the liver.

The feeding of sugar to phosphorus poisoned animals conserves and replenishes the hepatic glycogen and markedly diminishes the toxic effect of the phosphorus as shown by the mildness of the clinical symptoms, by the continued presence of glycogen in the liver, and by the very moderate degree of degenerative changes in that organ. The available data in the literature on the chemical, enzymatic and metabolic changes in chloroform poisoning, acute yellow atrophy and in eclampsia, are not so complete as for phosphorus poisoning, but from the facts at hand there is reason to believe that the differences are more quantitative than qualitative, and that the administration of sugar in these three conditions will have something of the same protective effect as in phosphorus poisoning.

An attempt has been made to arrive at some understanding of the mechanism of this protective action of sugar, or more correctly, of the conservation and replenishing of the supply of glycogen of the liver, in poisoning with phosphorus. It has been suggested that an adequate supply of glycogen may affect the state of the colloids of the cell, (1) by stabilizing the emulsion and preventing its "breaking" with the throwing out of droplets of fat; and (2) by reducing the permeability of the cells to the poison.

From studies of the literature there is reason to believe that a certain amount of carbohydrate is essential to normal protein metabolism. If it is not supplied in the diet it is obtained by increased breaking down of protein.

In phosphorus poisoning there is a diminution of the power of the body to carry on its normal oxidative processes, and, at the same time, there is an increased demand for readily oxidizable material. Fat cannot satisfactorily supply this augmented need in the presence of the reduced oxidizing power, because fat is twice as difficult to oxidize as carbohydrate. An attempt is made to meet this increased demand on the part of the liver by the transportation to it of fat which it is unable to burn, and by increased protein metabolism. It has been shown that the liver is able to burn its own glycogen more readily than glucose or many other substances supplied to it.

Many of the changes occurring in phosphorus poisoned livers are of the nature of an autolysis. The presence of glycogen in the liver appears to inhibit the autolysis of this organ in vitro, because it has been shown that autolysis begins more promptly, proceeds more rapidly and brings about a more complete degradation of the protein molecule in those livers which have been previously rendered glycogen-free by starvation of the animal, or by poisoning with phosphorus, or by the administration of thyroid extract.

It is believed, further, that the autolysis occurring in phosphorus poisoned livers is the result of a reversed action by those intracellular enzymes which, normally carry on the synthetic processes in protein metabolism. On account of changes within the cell, possibly the result of reduced oxidation, the equilibrium point in the enzyme reaction is displaced, and the normal function of the enzyme reversed.

The conservation and replenishing of the glycogen of the liver by the administration of sugar is believed, therefore, to protect the animal against phosphorus poisoning (1) by its effect on the colloids of the cell; (2) by furnishing carbohydrate which appears to be essential to the normal synthetic processes of protein metabolism; (3) by supplying the most easily oxidizable material to an organ whose oxidizing power has been reduced by the poison; (4) by inhibiting the process of autolysis in vivo; and (5) by preventing the reversal of the action of the intracellular enzymes.

It is believed that the facts here presented are sufficiently well substantiated to justify the opinion that the administration of sugar will prove to be an important therapeutic measure in phosphorus and chloroform poisoning in humans, in acute yellow atrophy of the liver, and, possibly, in eclampsia.

2431 South Dearborn Street.

EXPERIENCES WITH A RECENT EPIDEMIC OF MENINGOCOCCIC MENINGITIS AMONG A CHINESE CIVIL POPULATION *

PETER K. OLITSKY, M.D.
NEW YORK

During the past spring and early summer (1918) a study was made of an epidemic of meningococcic meningitis occurring among a civil population in a district in South China. Some of the results of the investigation have proved of sufficient interest to warrant their somewhat detailed description, in the hope that they may be of value to the medical officers of the Army and Navy combating epidemic meningitis.

GENERAL DESCRIPTION

The beginning of the epidemic is obscure. An epidemic of meningococcic meningitis heretofore among the Southern Chinese is practically unknown. Hence, when the first cases occurred, the proper diagnosis was not made. It was not until the second week in February that cases which were regarded previously as hemorrhagic smallpox were definitely diagnosed as epidemic meningitis. From that time until the first of June there were reported officially, 1,041 cases; but it is estimated that, including the "missed" cases, the actual number was nearer 2,500. Among the cases reported the mortality was 85 per cent. The epidemic continued until the first week of July; the largest number of cases occurred in March.

The source of infection is likewise indefinite. Although no epidemics have occurred in this district, yet it is reasonable to assume the occurrence of sporadic cases now and then. Indeed, there is a case on record which appeared five years ago. Again, in the Philippine Islands, which are on a direct trade route, there were reported about three years ago seventy cases, of which some yielded pure spinal cultures of the meningococcus. Furthermore, this district is a great gateway to the southern part of the Orient, and enormous numbers of persons pass through it; undoubtedly some are carriers of meningococci.

The locality has points of interest with reference to epidemic meningitis. In the first place, it is situated in the subtropical zone bordering on the tropical. In view of the extent of the epidemic, it becomes problematical whether this disease is one of the temperate

* From the Laboratories of the Rockefeller Institute for Medical Research.

zone. In the second place, the district is greatly overcrowded,[1] and in some quarters there exist living conditions quite analogous to those found in an overcrowded camp. Again, the population comprises for the most part poor people, passively resisting western methods, so that the epidemic was disseminated thickly on a good soil.

OBSERVATIONS ON TREATMENT

The following observations were made at a local hospital where none but Chinese were received. They are based on a total number of 417 patients. At this point it may be stated that in certain sections of China conservatism is still very strong and modern medicine is not favorably entertained. Hence the following statistics offer a sad but interesting study:

One hundred and four patients received neither serum treatment nor lumbar puncture. Of these, 84.6 per cent. died. This mortality corresponds with the general death rate for the disease throughout the district.

In 346 patients lumbar puncture was made from once to five times. Of these 54.1 per cent. died.

In 71 patients, lumbar puncture combined with the spinal injection of antimeningococcic serums having a low antibody content, was made. Of these, 45 per cent. died. These serums were on hand before my arrival.

(Taking at random 10 spinal cultures isolated from local cases, one serum agglutinated 5 of them, but 3 other specimens agglutinated from 2 to 3, usually in dilutions of 1:50. Flexner serum used as a control agglutinated 56 out of 59 similar cultures and usually in dilutions of 1:800.)

Several conclusions may be drawn from these records. First, the mortality is appalling when no treatment is given. Second, spinal tapping by itself, while showing an improvement in the death rate, is still far from a satisfactory therapeutic procedure. Third, the injection of a serum poor in quality offers only slightly better hope. Lastly, a serum low in agglutinin content is apt to be therapeutically ineffective.

When these methods of treatment are compared with the results recently obtained in civil and military practice by proper antimeningococcic serotherapy, it is certain that the employment of the latter is indicated at all times.

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1. The area of the district is 390 square miles. The official population is 535,100 and the population to the square mile 1,372. But 300,000 of these Chinese live in a small section of this area—a section about 3 miles long and one-half mile wide.

BLOOD CULTURE STUDIES

At present an extensive literature is being reported on the appearance of the meningococcus in the circulating blood. It is stated that the organism appears very early in the course of the disease. On this account the following blood cultures should prove of interest. Owing to certain difficulties no selection of cases could be made, but in this series of ten cases it happened that all were moribund, and in some instances the blood culture was made in the agonal period.

The blood cultures were made by inoculating from 2 to 3 c.c. of whole blood into each of two flasks containing 100 c.c. of veal infusion, 1 per cent. dextrose broth of 0.7+ acidity (phenolphthalein). Besides these flasks, about four or five plates of dextrose agar were poured, each containing 2 c.c. of blood. None of the plates was positive; a positive result was usually indicated by growth in one or both flasks. All cultures thus obtained were tested for type and corresponded with the spinal strain—the parameningococcus in all instances, as I will describe later.

Case 1, from whose spinal fluid parameningococci were isolated, was cultured on the fourth day of illness. The patient died two days later. The blood culture was negative.

Cases 2, 5, 6, 8 and 9 yielded no growth from the spinal fluids (except Case 2), but the appearance of the fluids was typical and they contained many gram-negative diplococci, intracellular and extracellular.

The five patients died two days after obtaining the culture, which was taken on the fifth day of illness, except in Case 6, when it was taken on the second day of illness. The cultures were negative in all cases.

In Cases 3 and 4 the patients were comatose on the fifth day of illness, when the culture was taken. They died the following day. The blood as well as the spinal fluid showed a growth of meningococci.

Cases 7 and 10 were similar in detail to Case 3, except that there was no coma or petechial rash, but death occurred the day following the taking of the culture, which was positive.

From these cases one observes that of the ten patients, four yielded positive blood cultures, but of the latter all died one day later. It is difficult to interpret the small number of findings; and the evidence is too scant to indicate whether the infection was primary or secondary in the blood in these cases. The specimens were taken antemortem and could be explained as antemortem meningococcal invasions of the blood. However, the results served to emphasize the desirability of intravenous therapy.

THE PREVAILING TYPE OF MENINGOCOCCUS AMONG PATIENTS

Spinal cultures in a pure state were obtained from 60 patients. Fifty-nine of the patients were examined at the height of the epidemic. Of these, 56 were para types and 3 irregular para types. The para type when tested corresponds with Type I of Gordon's (English) classification; the normal, with Type II; and the irregular para possibly with Type III. The para type agglutinations ranged from 1:50 to 1:800; the irregular para types showed the reactions in Table 1.

TABLE 1.—REACTIONS SHOWN BY IRREGULAR PARA TYPES

Culture Number	Normal Type Immune Serum	Para Type Immune Serum	Polyvalent Serum
021	1:50	1:400	1:800
080	1:100	1:400	1:400
052	1:50	1:200	1:200

From this we conclude that during the height of the epidemic almost 95 per cent. of the patients were infected by the same type of meningococcus, the para type.

The remaining case of the sixty patients examined occurred during July when the epidemic was on the wane and the incidence of the disease in a given section was relatively infrequent. It was remarkable that this patient should yield a normal or regular type meningococcus.

In this connection it is interesting to note that two of the cultures isolated three years previously in the Philippine epidemic were tested and found to be para types. Also an epidemic of meningitis in a city along the trade route from this district and occurring somewhat later, yielded nineteen para types out of twenty spinal cultures isolated. The inference is that during the height of the epidemic almost all the patients were infected with one type, the parameningococcus.

BACTERIOLOGIC STUDIES ON CARRIERS

(*a*). *Contact Carriers.*—No extensive work could be done on contact carriers at the height of the epidemic, as I arrived at the time of its subsidence. I shall, however, discuss the results obtained then by the local bacteriologists who made several cultures.

These cultures were made as routine examinations of contacts with patients for the presence of the meningococcus in the nasopharynx. It appears that the work was very carefully done; only a few cultures were examined at a time and all plate cultures contaminated with saliva were discarded. The criteria for the recognition of the meningococcus were (1) typical colony morphology, (2) absence of pigment, (3) acid production in dextrose and maltose and not in saccharose medium,

(4) typical morphology by Gram's stain, (5) ready emulsification of the growth of subcultures. No agglutination tests were made, however. Sheep serum water (1 part serum to 2 parts water) 20 per cent., agar was used for the plate cultures.

Examining once only contacts of several patients, it was found that:

71 Europeans	yielded 7 carriers	(9.8 per cent.)
133 Chinese	yielded 9 carriers	(6.7 per cent.)
204 examined	yielded 16 carriers	(7.8 per cent.)

The ratio of incidence of meningitis among the Europeans to the white population of the district was as 1 is to 1,250; in the case of the Chinese to the colored population as 1 is to 212 (if 2,500 is used as the basis for the number of cases). These cultures were taken at the height of the epidemic; the number of contact carriers in both series is lower than is usually the case.

One deduction, however, can be made, namely, that the carriers were more prevalent among the white population, notwithstanding the fact that very few of the latter were afflicted (4 to 2,500 of Chinese).

(*b*). *Noncontact Carriers.*—As no opportunity presented itself to continue the study of contact carriers, my attention was paid to a class which may be regarded only nominally as noncontact carriers. In the event of an epidemic of so great an extent as this one, a group can hardly be chosen as a representative noncontact class. It was finally decided that a local jail, in which no case of epidemic meningitis developed throughout the epidemic, would offer a suitable source for the determination of the number of carriers for comparative study.

In interpreting the results, these factors should be considered: The prisoners were in the main previous residents of the district. There was a daily average of 600 inmates. The length of confinement before the cultures were taken varied from a few days to several years. The prisoners were kept isolated in individual, well ventilated cells; there was no overcrowding. In brief, the sanitary conditions in the jail were better than in a great part of the Chinese community.

The following cultures were made on unlaked sheep blood, veal infusion agar with 1 per cent. dextrose (defibrinated blood 1 part, agar 15 parts). The cultures were taken from the nasopharynx with a West swab, care being taken to avoid contamination with saliva. The plates, each person's culture on a single plate, were incubated immediately after inoculation.

The total number of Chinese prisoners swabbed was	151
Plate cultures contaminated with saliva and therefore discarded were	21

A. Negative Cultures

1. Plate cultures showing no suspicious colonies.... 74
2. Those showing suspicious colonies which failed to grow characteristically..... 19
3. Those showing suspicious colonies which were proven negative by agglutination ("flavus agglutination") 3
4. Those showing suspicious colonies which subquently grew at room temperature..... 2

Thus making a total of 98 negative cultures.

B. Positive Cultures

1. Plate cultures showing typical colonies, consisting of gram-negative diplococci of characteristic morphology, but which failed to grow on subculture. There were usually two or three colonies transplanted. On the basis of previous experience it was thought proper to include these with the positive cultures. They were as shown in Table 2.

TABLE 2.—Plate Cultures Showing Typical Colonies

Serial Number	Age (Years)	Time in Jail	Degree of Infection*
7	20	1 mo.	±
46	25	2 mos. 9 days	+
70	30	2 mos.	±
97	27	1 mo. 5 days	±
132	28	5 mos. 14 days	±

* The term "degree of infection" is used to signify the relative number of colonies in the plate cultures. This gives an indication as to the extent of the infection of the nasopharynx. Hence ± signifies an occasional colony; +, few colonies; ++, numerous colonies of meningococci, but other organisms predominating; +++ meningococcus colonies predominating; and ++++, a pure culture of the meningococcus.

2. Plate cultures showing typical colonies consisting of gram-negative diplococci of characteristic morphology; producing acid in dextrose and maltose, but not in saccharose medium; showing no growth at room temperature, but a typical growth on subculture (ready emulsification, no pigment, mucoid, etc.); showing no agglutination in saline or normal horse serum (1:50) controls, and no agglutination with type or polyvalent antimeningococcic serum (Table 3).

TABLE 3.—Plate Cultures Showing Inagglutinable Cultures

Serial Number	Age (Years)	Time in Jail	Degree of Infection
56	31	2 mos. 7 days	++
75	27	14 days	+
76	30	11 days	++
100	22	2 mos. 7 days	+
124	34	3 yrs. 5 mos.	+

3. Plate cultures showing colonies having the characteristics mentioned under 2, but possessing definite agglutination reactions with polyvalent or type serum or both (Table 4).

TABLE 4.—Colonies from Jail Controls Possessing Definite Agglutination Reactions

Serial No.	Age (Years)	Time in Jail	Degree of Infection	No. of Colonies Exam.	Agglutination Reactions (Dilutions of 1:50 and 1:100)*	Type
1	53	1 yr. 4 mos.	±	1	Poly., ++; normal, ++; para, 0	Normal
20	21	1 mo. 6 days	±	2	Poly., ++; normal, 1:50, +; para, ++	Irregular para
21	34	1 mo. 29 days	++	1	Poly., ++; normal, ++; para, 0	Normal
22	29	11 days	+++	1	Poly., ++; normal, 1:50, +; para, ++	Irregular para
28	27	3 mos.	+	1	Poly., ++; normal, 0; para, 0	Irregular
50	23	1 mo. 27 days	±	1	Poly., ++; normal, ++; para, 0	Normal
82	20	17 days	±	1	Poly., ++; normal, ++; para, 0	Normal
84	46	7 days	±	2	Poly., ++; normal, ++; para, 0	Normal
88	55	1 mo. 9 days	+	2	Poly., ++; normal, ++; para, 0	Normal
89	34	1 mo. 6 days	+++	2	Poly., ++; normal, ++; para, 1:50, +	Irregular normal
102	39	19 days	±	1	Poly., ++; normal, 0; para, 0	Irregular
112	30	29 days	±	1	Poly., ++; normal, 0; para, ++	Para
117	20	1 mo. 14 days	++	1	Poly., ++; normal, 0; para, 0	Irregular
122	38	2 mos. 14 days	±	2	Poly., ++; normal, ++; para, 0	Normal
123	38	2 mos. 14 days	+	1	Poly., 0; normal, ++; para, 0	Normal
135	32	1 mo.	±	1	Poly., 0; normal, ++; para, 0	Normal
145	30	1 yr. 11 mos.	±	1	Poly., ++; normal, ++; para, 0	Normal
151	16	4 mos. 20 days	+	3	Poly., ++; normal, ++; para, 0	Normal

* ++ indicates complete agglutination in a dilution of 1:100; +, incomplete agglutination in the dilution stated.

4. To the last mentioned may be added plate cultures yielding colonies indistinguishable from spinal strains but having no definite type reactions and showing a definite sedimentation along with a slight

agglutination with Flexner's serum, but not with normal horse or type serum. As they all reacted similarly, these cultures were placed in one group, as given in Table 5.

TABLE 5.—Group of Cultures Showing Sedimentation and Slight Agglutination with Flexner's Serum

Serial No.	Age (Years)	Time in Jail	No. of Colonies Examined	Degree of Infection
41	25	24 days	1	±
106	29	1 mo. 28 days	1	±
114	45	1 mo. 1 day	1	±
131	39	6 days	1	±

Summarizing the results of the positive cultures, we find that there were:

Normal types (corresponding to Type II of the English classification)	11
Para type (corresponding to Type I)	1
Irregular normal type	1
Irregular para type	2
Irregular (no agglutination with normal or para only with polyvalent serum)	7
Inagglutinable types	5
Cultures impossible to agglutinate as no growth was obtained	5
Total	32

Therefore, notwithstanding the fact that no cases occurred in the jail, we find in the series of prisoners examined that 24.6 per cent. harbored meningococcus-like organisms in the nasopharynx.

The age of the inmate apparently had no bearing on the carriage of the meningococcus.

The length of time of detention in the jail previous to the taking of the culture is summarized in Table 6.

TABLE 6.—Time in Jail Previous to Making Cultures

Number of	Up to 1 Month	1 to 3 Months	3 to 6 Months	6 to 12 Months	After 1 Year
Negatives:	32	31	10	13	12
Positives:	9	18	3	0	2

It will be noted from Table 6 that most of the inmates showing positive cultures had been confined up to three months. The epidemic was four to five months in its course when the cultures were taken. The inmate who carried the para type — the type prevailing in the epidemic — was confined twenty-nine days. Two others, infected with irregular para types — also found in a very few patients — were confined eleven and thirty-six days previous to the culture. It appears, then, that the older prisoners, under the conditions of life in this

prison, apparently become to a great extent free from the meningococcus. It also appears that the few inmates who harbor the type prevailing in the epidemic have been recently confined.

With reference to the degree of infection, it is interesting to note that most of these carriers harbored only a few or occasional organisms. In 4 of the 32 carriers, however, they were numerous (++), and in two the meningococcus was the predominating organism (+++). The only inmate who carried a para type — the prevailing type of the epidemic — was lightly infected.

In several instances, more than one colony of the same plate culture was examined for type. The results confirm the already established fact that the meningococci isolated from the same nasopharynx are usually of the same type.

Finally, an analysis of the types found shows that only one inmate yielded a para organism. On the other hand, 34.3 per cent. of the carriers yielded the normal type and the remainder (excluding 5 in whom no serologic tests could be made) or 50 per cent. of the carriers, harbored irregular or inagglutinable types.

In conclusion, the results of the swabbing of a number of inmates of the jail show that 24.6 per cent. were carriers of organisms indistinguishable from meningococci; that these carriers yielded types which, generally, were not found among the patients, namely, normal, irregular or inagglutinable organisms. Also, the percentage of carriers in the jail was higher than that demonstrated by others among the contacts with patients during the epidemic. Finally, among the 600 or more prisoners, no case of epidemic meningitis developed.

OTHER EPIDEMIOLOGIC FACTORS

In view of the fact that the following observations were made during an epidemic in a civil population, they are of interest for comparison with the experiences obtained in the Army and Navy.

(*a*). *The Influence of Age and Sex.* — From the local hospital records of 417 patients it will be noted that the ages of the patients ranged from 4 months to 59 years; the average age was 22.68 years. From the record of the Medical Officer of Health, based on the first 750 cases, mainly fatal, the great peak of the curve of age incidence is from infancy to 5 years; a lesser curve is seen at 17½ years.

The number of male cases at the local hospital was over twice that of the females; the official records show males to have been only slightly in excess of females. These figures when compared with the numbers of the sexes of the normal population, would be corrected so that the number of male cases is in excess of female only among the young adults.

Therefore, the susceptible elements of the population are the children, with no definite sex preponderance, and young adult males.

(*b*). *Influence of Meteorologic Conditions.*—The temperature, the mean as well as the wet-bulb (temperature of saturation), had a marked influence on the incidence of the disease. In general terms it may be stated that when there occurred a sudden drop of temperature, the number of cases increased, and when there was a rise in temperature, the incidence of epidemic meningitis declined. From a chart prepared by the Medical Officer of Health, one may formulate a table to show this relationship (Table 7).

TABLE 7.—Fluctuation of Number of Cases with Temperature Variations

Date	Degree of Drop (Temp. of Saturation)	No. of Cases (Increase Over Previous Low Level)	Time Elapsed Between Drop of Temp. and Increase of Cases
2/14-2/18	About 13° C.	11	4 days
2/27-3/ 2	About 11° C.	8	4 days
3/10-3/12	About 9° C.	4	2 days
3/25-3/27	About 12° C.	15	5 days
4/ 9-4/11	About 14° C.	5	5 days

On the other hand, the effect of a rise of temperature on the incidence of the disease can be formulated as in Table 8.

TABLE 8.—Effect of Rise of Temperature on the Incidence of the Disease

Date	Degree of Rise (Temp. of Saturation)	No. of Cases (Decrease Over Previous Low Level for Week)	Time Elapsed Between Rise of Temp. and Decrease of Cases
2/18-2/27	About 20° C. (gradual rise)	8	8 days
3/15-3/24	About 17° C. (gradual rise)	4	8 days
3/31-4/ 9	About 13° C. (gradual rise)	4	4 days

Subsequently, as the temperature rose there was a corresponding decrease in the number of cases.

From the above it will be seen that as a rule about four days after a drop in temperature there was a great increase in the number of cases reported. The correspondence of this number with the number of days of the incubation period of epidemic meningitis is quite suggestive.

The influence of sunshine has also a relationship to the number of cases. For example, the period from February 28 to March 3 (four days) showed only 5½ hours of sunshine. Three days later, 23 cases were reported, the greatest number reported for a day with one exception. There was practically no sunshine for four days from March 26; on the fourth day after this period, 21 cases were reported, although there were only from 5 to 15 cases for the previous week. Thus, the lack of sunlight shows a relationship to the increase in the number of cases.

It is doubtful whether humidity or rainfall by themselves have any bearing on the number of cases. January and February were extremely dry months. Indeed, the rainfall was considerably below the average for several years. Yet February saw increasing numbers of cases, and March with a relatively small amount of rainfall, the greatest number. Similarly with the humidity; increasing humidity bore no relation to the number of patients.

(*c*). *Pre-epidemic Infections.*—It is a matter of common knowledge among the practitioners, although no official records are available, that many cases of sore throat were prevalent in the district during the period preceding the epidemic (November and December, 1917). The ailment was not severe, but at times it resembled influenza. Its distribution, however, was widespread.

On the other hand, there is no evidence of any increase in the incidence of measles or mumps.

(*d*). *Migration of the Population.*—The effect of emigration[2] from the district on the widespread dissemination of the disease is clearly shown. A few weeks after the outbreak cases were noted (and in most instances for the first time) in five cities along the trade routes north and south of the district. The cases numbered from a few to sixty, and in one instance nineteen out of twenty cases yielded a parameningococcus, the prevailing type.

The influence of immigration, on the other hand, on the continuation of an epidemic suggests itself. The entrance of a new, susceptible element of population from a noninfected district and its existence in close contact with the disease should increase the number of cases. Or a disturbance of the ratio of insusceptibles of the native population is created, offering new soil for an outbreak. The extent of immigration may be inferred from the records of 1916 (72,405) and 1917 (98,232).

(*e*). *The Habits of the Natives.*—Certain infringements of the rules of hygiene which may have a special relationship to the spread of the disease are continually practiced by the Chinese of this district. The most flagrant are in connection with the street restaurants. The edibles are exposed to the open air and served in dishes which are not cleansed between the servings, so that what is in one man's mouth may be directly conveyed to another's The other infringements relate to the use of common towels and drinking cups; the drying and sorting of food on the highways which are covered with expectoration.

2. The activity of emigration may be judged from the records for 1916 and 1917, namely, 117,653 and 96,298, respectively.

(*f*). *The Overcrowding of the Population.*—As the epidemic was limited to a great degree to the overcrowded sections, it is important to describe this condition in detail.

Three causes operate to effect overcrowding. In the first place, there are too many houses on too small a space. Structures are in close proximity, separated from one another by narrow, chimney-like areaways. The lanes and areaways are so narrow, especially in proportion to the height of the houses, that not only ventilation but also light becomes deficient. The air is likewise damp almost all the time, from the wetness of the passageways.

In the second place, there are no arrangements for ventilation within the houses. For example, a house having a content of 5,818 cubic feet, has a window area of 51.8 square feet. One of the windows opens into a narrow street, the other into an oblong areaway of the diameter of a large steamship funnel. Besides, to avoid thieving or intrusion, the windows and doors are usually bolted, making the air very foul.

In the third place, there are too many inmates within a house. A floor of a dwelling is divided into boxlike compartments, called cubicles. There are from four to six cubicles to a floor. They are solidly partioned off and the entrance curtained, so that no air whatever is admitted. This is the case as a rule, although exceptions may be found in the first or last cubicle. A typical cubicle on investigation revealed that its content is 336 cubic feet of unventilated air space. This cubicle is the dwelling of six persons. It is a frequent occurrence to find twenty persons existing on a floor space of 270 square feet.

The tendency of the Chinese to overcrowd is noted throughout the community. The market is crowded, so are the streets, the street cars, the lodging houses, native schools and matsheds.

Hence, there are too many houses on too small an area; there are no sanitary arrangements for light and air within the houses; there are too many persons living within the houses, and there is an innate gregariousness — all causing dense overcrowding.

(*g*). *Geographical Distribution of Cases.*—It is of interest to note that while it was of rare occurrence that more than one member of a family was afflicted, yet the greatest number of cases occurred in limited areas. Briefly, these areas comprised the most overcrowded sections. Indeed, in proportion to the same number of inhabitants, sparsely settled or less crowded areas yielded none, or an occasional case. Within the crowded district there are sections still more densely congested; the percentage of incidence to the population in the latter areas was three times that of the former.

EPIDEMIOLOGIC CONCLUSIONS AND DISCUSSION

The conditions prevailing in the district have been stated, and an attempt will be made to draw from them epidemiologic conclusions. The conditions usually prevailing during a pre-epidemic period occurred here as well, namely, an extremely cold winter combined with a large number of cases of pharyngitis, bronchial affections, and possibly influenza. In view of previous experience, these circumstances are favorable for the development of cases of epidemic meningitis, but they do not explain the great spread of the disease in the district, especially in certain sections.

(*a*). *The Influence of Overcrowding.* — The factor of greatest importance in the dissemination of the disease is the extensive overcrowding. The bases on which this conclusion rests are:

1. The meteorologic conditions.
2. The relative incidence of the disease in crowded and in uncrowded sections.
3. The bacteriologic evidence obtained from the patients.
4. The relation of the carrier.

It has been demonstrated above that *meteorological factors* have an important relationship to the dissemination of the disease: cold weather, especially in the absence of sunshine, resulted in an increase in the number of cases.

The effect of cold weather on the population is remarkable. As soon as it is felt, the natives abandon the habit of sleeping out in the open streets and highways, and literally swarm in the cubicles. These cubicles, as already stated, are boxlike compartments completely closed in and absolutely unventilated.

The mechanism of dissemination of the organism is more or less direct. It "consists in the ejection of the nasopharyngeal secretions into the surrounding atmosphere. This ejection does not take place during ordinary breathing, and little, as a rule, during quiet speaking. But in loud speaking and particularly in coughing, sneezing, hawking, and spitting, the secretions may be sprayed and scattered widely."[3] Should one inmate harbor the meningococcus, it will be soon distributed over all the others of the dwelling. Thus, while the meningococcus is a very fragile organism and succumbs easily in external nature, in this condition of overcrowding and close contact induced by the cold weather, a general distribution is favored.

It is logical to assume that in a dark and damp atmosphere the viability of the organism is prolonged, favoring as well its spread from one to another.

3. Flexner, Simon: Mode of Infection, Means of Prevention and Specific Treatment of Epidemic Meningitis, J. A. M. A., **69**:639, 1917.

The relative incidence of the disease in crowded and uncrowded sections adds further evidence. As shown in the foregoing, the disease took its greatest toll from the crowded districts. Among the foreign element an occasional case developed; among the Chinese it was common. Yet epidemic meningitis is not a disease peculiar to the Chinese.

On the other hand, in a neighboring city where there are four times as many Chinese, but not so densely settled, and where there is no cubicle system of dwelling, but the people live in ventilated rooms, not a single case developed among the natives.

The *bacteriologic evidence* obtained from patients is suggestive of the spread of the disease by close contact. Almost all (95 per cent.) the patients examined were infected with one type, the parameningococcus. As other types were found in the community, it is reasonable to assume (among other things) the rapid communicability of this type one to another — a result of the great density of the population.

The *relation of the carrier* in comparison with overcrowding was studied in the inmates of the jail. The results show that 24.6 per cent. of the 130 inmates examined were carriers of various types of meningococci. Yet at this institution not a single case developed. The important fact is that there are isolated cells there and no crowding.

Also among contacts, Europeans showed 9.8 per cent., while Chinese showed 6.7 per cent. of carriers. Yet the Chinese by far outnumbered the European cases.

This evidence leads to the conclusion that overcrowding is the important factor in the spread of the epidemic. At the same time, one must regard the carrier as a *sine qua non* as regards the source and the agent of transmission of the disease.

It appears, however, that the actual numbers of carriers present have only little epidemiologic significance. It is the type which is carried that is significant. Patients yield para types; the prisoners, although within the epidemic area but among whom no case develops, yield practically only normal, irregular, and inagglutinable types. The question of their relationship is still under investigation. However, it appears to favor the view recently stated by Flexner[4] that in healthy carriers one type (the saprophytic), while in the epidemic cases another (the pathogenic), prevails.

(*b*). *Other Factors Contributing to the Continuance of the Epidemic.*—The epidemic having gained headway, it is possible that its continuance depended on the constant migration and the unhygienic habits of the natives: the many ways prevailing in the Chinese community of distributing nasopharyngeal secretions from one to another.

4. Flexner, Simon: Control of Meningitis, J. A. M. A., **71**:638, 1918.

RECOMMENDATIONS FOR PREVENTION

The local conditions led to a formulation of the following means of prevention. The efficacy of these means cannot be judged until the next cold season will have passed. In outline form they are:

(*a*). The education of the Chinese in order to effect an active cooperation with the health and sanitary authorities.

(*b*). The prevention of overcrowding.

(*c*). The prevention of droplet infection by the instruction of the principles of personal hygiene and the employment of masks.

(*d*). The detection and treatment of contact carriers including the isolation of the "dangerous" carrier (one who harbors numerous meningococci, particularly of the same type as the patient).

(*e*). The isolation of the patient.

(*f*). Although still in an experimental stage, the use of preventive inoculation of an antimeningococcic vaccine.

CONCLUSIONS

In four of ten moribund cases of epidemic meningitis, the meningococcus was found circulating in the blood. It is difficult to interpret these findings with regard to the theory that blood invasion is primary or secondary, as in these cases the results were obtained antemortem. However, it emphasizes the need of intravenous combined with the intraspinous methods of treatment.

Serums having a low agglutinin content were therapeutically ineffective.

About 95 per cent. of the patients were infected with one type, the parameningococcus; the remainder with the irregular para type.

In a series of prisoners, who lived under hygienic conditions, 24.6 per cent. were found to be carriers. Of these, 50 per cent. yielded irregular or inagglutinable types and 34.3 per cent. the normal type, and only one person the para type. Although the jail is within the epidemic area, not a single case of meningitis developed therein.

These and other facts already stated have led to the conclusion that dense overcrowding of population and the presence of a pathogenic type of meningococcus, rather than the actual numbers of healthy carriers of various types of the organism, are the causes of the great spread of this epidemic.

BIOCHEMICAL STUDIES OF PNEUMONIC EXUDATES

WITH SPECIAL REFERENCE TO THE MECHANISM OF THE CRISIS IN PNEUMONIA *

STUDIES IN PNEUMONIA. X

CHARLES WEISS, PH.D.
PHILADELPHIA

In a previous communication[1] we demonstrated the following properties of pneumonic lung exudates:

1. Salt solution extracts of human lungs in the stage of gray hepatization in pneumococcus lobar pneumonia are more toxic for experimental animals than similar extracts of normal lung tissue. The method of extraction influences the toxicity of both extracts.

2. Lethal doses of extracts of both pneumonic and normal lung tissue injected intravenously usually produce anaphylactic-like symptoms.

3. Sterile extracts of pneumonic lung tissue of dogs removed forty-eight hours after intrabronchial insufflation of virulent pneumococci are more toxic than similar extracts of consolidated lung following intrabronchial insufflation of sterile aleuronat in suspension and both of these are somewhat more toxic than extracts of equal weights of normal dog lung.

4. The toxicity of extracts of normal and pneumonic lung is decreased by heating, drying and filtration through porcelain filters.

5. Extracts of human pneumonic lungs in gray hepatization are hemolytic for guinea-pig cells, whereas similar extracts of normal human and dog lungs and of consolidated dog lungs following the intrabronchial insufflation of virulent pneumococci and sterile aleuronat, are generally nonhemolytic. The hemolytic activity of these extracts is neutralized by horse antipneumococcus serum as well as by normal rabbit serum; it is reduced by heating and drying and usually completely removed by porcelain filtration.

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* From the Department of Physiological Chemistry and the McManes Laboratory of Experimental Pathology of the University of Pennsylvania.

* Part of a dissertation presented by the writer for the degree of Doctor of Philosophy, University of Pennsylvania, 1918.

* Aided by the Fels grant for research in pneumonia.

1. Weiss, C., Kolmer, J. A., and Steinfield, E.: The Toxicity of Pneumonic Lungs, J. Infect. Dis. **22**:469, 1918.

6. Extracts of human pneumonic lung in gray hepatization inhibit the agglutinating activity of antipneumococcus serum.

The present investigations deal with further biologic and chemical analyses of the toxic substances in pneumonic exudates.

METHOD OF STUDY

The method of study consisted in anaphylactic sensitization and intoxication of guinea-pigs with various proteins derived from normal and pneumonic lungs, exudates, serums, etc. It has been recently shown by White and Avery[2] and particularly by C. L. A. Schmidt[3] that the anaphylactic test is a very accurate one for the study of the biologic specificity of proteins. These anaphylactic tests were supplemented by studies of the comparative toxicity for rats and rabbits of the various proteins isolated. Their hemolytic activity was also determined. For the sake of accuracy all tests were done either in duplicate or in triplicate.

TABLE 1.—RESULTS OF ANAPHYLACTIC TESTS WITH HUMAN NORMAL AND PNEUMONIC EXUDATES

Weight of Guinea-Pig, Gm.	Sensitizing Substance	Intoxicating Substance	Results*
235	Pneumonic exudate	Pneumonic exudate	+++
254	Pneumonic exudate	Normal lung exudate	—
206	Normal lung exudate	Normal lung exudate	+++
222	Normal lung exudate	Pneumonic exudate	—

* In this and the following tables +++ = very severe anaphylactic shock, with death and typical necropsy findings; ++ = very severe anaphylactic shock with recovery; + = moderate shock with recovery; — = no reaction.

In our preliminary experiments we worked with whole phenolized exudates prepared according to the technic given in our first paper.[1] The tests were conducted as follows:

One series of guinea-pigs was sensitized by intraperitoneal injection of the exudate obtained from normal lung and another series with that of pneumonic lung. Fourteen days later half of each series were intoxicated by intravenous injections of normal and the other half of pneumonic lung exudate.

The reactions obtained in each series were clearly specific. Guinea-pigs sensitized with a dose of 0.2 c.c. (corresponding to 40 mg. of dried exudate) per kilogram reacted when injected intravenously

2. White, B., and Avery, O. T.: Some Immunity Reactions: The Biological Reactions of the Vegetable Proteins, III, J. Infect. Dis. **13**:103, 1913.

3. Schmidt, C. L. A.: Studies on the Formation and Antigenic Properties of Certain Compound Proteins, Univ. of California Pub. in Path. **2**:157, 1916.

with a similar dose of the corresponding substance. Control animals receiving this dose on primary intravenous or intraperitoneal injection showed no symptoms. The results are shown in Table 1.

After having thus demonstrated the existence of a specific sensitizing substance in the exudates of human pneumonic lungs in the stage of gray hepatization, we set out to determine its chemical and biologic nature: its specificity, its relation to pneumotoxin and pneumococcus protein, to the normal serum proteins, and the proteins of the lung tissue substance itself.

For this purpose anaphylactic tests were conducted according to the scheme shown in Table 2.

TABLE 2.—RESULTS OF ANAPHYLACTIC EXPERIMENTS WITH HUMAN PNEUMONIC EXUDATES *

Weight of Guinea-Pig, Gm.	Sensitizing Substance	Intoxicating Substance	Results
220	Pneumonic exudate	Emulsion of heat-killed pneumococci	—
203	Emulsion of dead pneumococci	Pneumonic exudate	—
470	Pneumonic exudate	Pneumotoxin	++
265	Pneumotoxin	Pneumotoxin	+++
240	Normal human serum	Serum of toxic pneumonic patient	+++
240	Normal human serum	Pneumonic exudate	++
203	Antipneumococcus horse serum	Pneumonic exudate	—

* Sensitizing doses were 0.2 c.c. per kilogram. Intoxicating doses were somewhat less and always much below the minimum lethal dose, as determined by control injections.

The following facts are evident from these experiments:

1. Pneumonic exudate contains normal serum proteins and pneumotoxin.

2. Pneumonic exudate contains neither undigested pneumococcus protein nor albumin derived from lung tissue which possesses sensitizing powers.

With the object of studying the chemical nature of the pneumonic exudate under more controlled conditions, we sensitized guinea-pigs with the serums and lung exudates of dogs removed forty-eight hours after intrabronchial insufflation of virulent Type I pneumococci after the method of Lamar and Meltzer,[4] and also with similar material obtained from normal dogs. A protocol of these experiments is of dogs insufflated with virulent pneumococci. This observation is in given in Table 3.

The following points are to be observed:

4. Lamar, R. V., and Meltzer, S. J.: Experimental Pneumonia by Intrabronchial Insufflation, J. Exper. M. **15**:133, 1912.

1. Pneumotoxin is demonstrable in the lung exudate and serum accord with our previous findings with the use of an intracutaneous skin test.[5]

2. During the forty-eight hour interval between insufflation with virulent pneumococci and death of the animal by chloroform no specific sensitizing proteins (other than pneumotoxin) are developed in the exudate.

3. Pneumonic exudate contains *normal* serum proteins, leukocytes and blood fibrin.

TABLE 3.—Results of Anaphylactic Experiments with Material Obtained from Dogs Suffering with Experimental Lobar Pneumonia

Weight of Guinea-Pig, Gm.	Sensitizing Substance	Intoxicating Substance	Results
210	Normal lung exudate	Pneumonic lung exudate	++
205	Pneumonic lung exudate	Normal lung exudate	+++
225	Pneumonic lung exudate	Pneumonic lung exudate	+++
215	Pneumotoxin	Pneumonic lung exudate	+
215	Pneumotoxin	Serum pneumonic dog	++
265	Pneumotoxin	Pneumotoxin	+++
200	Pneumonic lung exudate	Serum normal dog	++
220	Pneumonic lung exudate	Serum pneumonic dog	+++
230	Normal dog leukocytes	Pneumonic lung exudate	++
260	Pneumonic lung exudate	Normal dog blood fibrin	+++
215	Normal dog blood fibrin	Pneumonic dog blood fibrin	+++
185	Serum pneumonic dog	Serum normal dog	+++

The lung exudate of dogs insufflated with virulent pneumococci is therefore similar to exudates obtained from cases of lobar pneumonia in man. It seems probable that the specific sensitizing protein present in the human gray hepatized lung is a product of the prolonged digestive action of the enzymes of the exudate on the normal serum albumin which is a constituent of the exudate. Müller's[6] observation that the enzymes of the pneumonic exudate are foreign to the lung and our own finding (see following) that the albumin obtained from pneumonic exudates is, judging by the specific anaphylactic reaction and higher toxicity, more or less different from the albumin obtained from a normal human lung exudate, are in accord with this view. This problem requires further experimental study and will be discussed more fully later.

5. Weiss, C., and Kolmer, J. A.: A Skin Reaction to Pneumotoxin, Proc. Soc. Exper. Biol. and M. **15**:93, 1918; J. Immunol. **3**:395, 1918.

6. Müller, F.: Ueber die Chemischen Vorgänge bei Lösungen der Pneumonie, Verhandl. d. Naturforsch. Gesellsch., Basle **13**: 1912.

It was deemed advisable to confine our studies to human pneumonic exudates. Preliminary experiments showed that there probably exists no qualitative difference in the amino-acid content of exudates of normal lungs and pneumonic lungs in the stage of gray hepatization. All uniformly gave the biuret, Millon's xanthoproteic, Hopkins-Cole and Heller's test. We therefore prepared from these exudates albumins, globulins and albumoses, and studied their specificity by anaphylactic sensitization of guinea-pigs and their comparative toxicity on primary injection into white rats and rabbits.

Technic of Fractionating Exudates.—Human pneumonic lungs in the stage of gray hepatization and normal lungs (showing no involvement or only a slight degree of postmortem congestion of the posterior lobes) were obtained immediately after necropsy. The tissue was cut into small pieces, washed free from blood with running water, mixed with salt and strained through thick sterile cheesecloth. The thick viscid fluid thus obtained was centrifuged for one hour at very high speed in a powerful electric centrifuge and the clear supernatant fluid was used for analysis.

The globulins were precipitated by the method of Banzhaf.[7] An equal volume of saturated ammonium sulphate solution was added and the precipitation was allowed to continue over night. The precipitate was centrifuged, washed with half saturated ammonium sulphate, taken up in distilled water and reprecipitated as before. It was again washed, dried at 20 C. by means of an electric fan, pressed between several layers of thick filter paper in a Buchner press, but not dialyzed. Dialyzation was deemed impracticable owing to the small amount of precipitate obtained. Control experiments showed that the retained ammonium sulphate in no way vitiated the results of the experiments. Chloroform and toluol were used throughout these analyses to prevent putrefaction.

The albumins were precipitated by complete saturation of the filtrate with crystals of ammonium sulphate ($(NH_4)_2SO_4$). This precipitation was permitted to continue over night in the presence of 1 per cent. acetic acid. The precipitate was centrifuged, redissolved and reprecipitated in a manner similar to the technic described. It was finally pressed and dried.

The albumoses were prepared as follows: The acid albumins were dissolved in physiologic sodium chlorid solution containing 0.5 per cent sodium carbonate (Na_2CO_3). The solutions were now strongly acidified with acetic acid, boiled and coagulated. The filtrates were regarded as containing the albumoses.

Reference is made in Table 4 to albumins from pneumonic lung tissue; for the preparation of these the lungs were entirely freed from exudate by grinding first in a meat chopper, then with quartz sand in a mortar, and finally pressed in a Buchner press with a force of 350 kg. per square centimeter. The exudate-free tissue was now thoroughly and rapidly dried by means of an electric fan at room temperature. The dry powdered tissue was shaken with distilled water for several hours to dissolve the proteins. After removal of the sand and other insoluble material by centrifugalization, the globulins and albumins were precipitated, using the technic described.

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7. Banzhaf, E. J., and Famulener, L. W.: The Proteins in the Serum of Goats Immunized Against Diphtheria, Collected Studies, Bureau of Laboratories, Dept. of Health, New York **8**:208, 1914-1915.

Pneumotoxin (endocellular toxin of virulent Type I pneumococci) was prepared after the method of Cole[8] as described by Cohen, Weiss and Kolmer.[9] The various proteins mentioned, including pneumotoxin and pneumonic exudates, gave all the required color reactions for proteins. The exudate-free lung tissue did not react to the Hopkins-Cole test for tryptophan.

The protocols of anaphylactic experiments conducted with the various proteins thus prepared are given in Table 4.

TABLE 4.—Results of Anaphylactic Experiments with Globulins and Albumins from Normal and Pneumonic Lungs*

Weight of Guinea-Pig, Gm.	Sensitizing Substance	Intoxicating Substance	Results
215	Globulin normal exudate	Globulin pneumonic exudate	+++
245	Globulin pneumonic exudate	Globulin normal exudate	++
425	Albumin pneumonic exudate	Albumin normal exudate	—
380	Albumin normal exudate	Albumin pneumonic exudate	—
199	Albumin normal lung tissue	Albumin normal lung tissue	+++
204	Albumin normal lung tissue	Albumin pneumonic lung tissue	+++
184	Albumin pneumonic lung tissue	Albumin pneumonic lung tissue	+++
299	Albumin pneumonic lung tissue	Albumin normal lung tissue	++
224	Globulin pneumonic lung tissue	Emulsion dead pneumococci	—
225	Albumin pneumonic lung tissue	Normal human active serum	+++
171	Egg albumin (Merck)	Albumin pneumonic lung tissue	—
235	Pneumonic exudate	Albumin pneumonic lung tissue	—
245	Pneumonic exudate	Albumin normal lung tissue	—

* Sensitizing dose was 0.1 gm. per kilogram in 1 per cent. solution in saline intraperitoneally. The intoxicating dose was somewhat less and always much below the minimum lethal dose, as determined by control injections.

The following points are to be observed:

1. The globulin fractions of human pneumonic and normal lung exudates are identical.

2. The albumin fraction of the pneumonic exudate possesses *marked* specificity and is related to the normal serum proteins.

3. Albumin from pneumonic lung tissue (freed from exudate) possesses no specificity and is also related to the normal serum proteins.

The validity of these conclusions was corroborated by the following observations on the comparative toxicity of the various lung proteins:

1. Globulins of both pneumonic and normal lung exudates are not toxic, producing no erythema or edema when injected intracutaneously into guinea-pigs in doses of 0.1 c.c. of a 1 per cent. solution in physi-

8. Cole, R.: Pneumococcus Hemotoxin, J. Exper. M. **20**:346, 1914.

9. Cohen, S. S., Weiss, C., and Kolmer, J. A.: On the Toxic Substances from Virulent Pneumococci, J. Infect. Dis. **22**:476, 1918.

ologic sodium chlorid solution, after the method of Wells and Hedenburg.[10]

2. Globulin of pneumonic lung (freed from exudate) is not toxic on intravenous injection into rabbits in doses of 5 mg. per kilogram (dissolved in physiologic sodium chlorid solution).

3. Albumins from normal and pneumonic lungs (freed from exudate) possess the same degree of toxicity for rats on intraperitoneal injection.

4. Albumin from exudate of pneumonic lung is far more toxic than similar albumin from exudate of normal lung, when injected intraperitoneally into white rats.

TABLE 5.—COMPARATIVE TOXICITY OF ALBUMINS FROM NORMAL AND PNEUMONIC LUNGS (FREED FROM EXUDATE) *

Normal Lung			Pneumonic Lung		
Weight of Rat, Gm.	Size of Dose, Gm. per Kg.	Result	Weight of Rat, Gm.	Size of Dose, Gm. per Kg.	Result
64	1.0	Dyspnea; convulsions; died, one hour	67	1.0	Dyspnea; convulsions; died, 10 minutes
57	0.6	Toxic symptoms; recovered, two hours	53	0.6	Sick; recovered, two hours
59	0.4	Slight symptoms; recovered	71	0.6	Slight symptoms; recovered

* Rats were injected intraperitoneally with 2 per cent. solutions of the albumins in normal salt.

5. Albumoses from exudate of pneumonic lung are exceedingly more toxic than similar albumoses from normal lung exudate.

In this connection a word must be said as to the specificity and marked toxicity of the albumins obtained from pneumonic exudates. It must be remembered that complete saturation with ammonium sulphate precipitates not only the albumins but also toxic albumoses and antitrypsin, as shown by Cathcart.[11] It is probably the digestive action of the trypsin (united with the antitrypsin) of the pneumonic exudate which is responsible for much of the toxicity and specificity of the albumins. The globulin fraction, as Cathcart showed, does not behave similarly, hence their nonspecificity and nontoxicity. The presence of the toxic albumoses does not vitiate our results, since they do not produce anaphylaxis, and we have made control studies of their comparative toxicity when free from albumins.

These results are shown in Tables 5 and 6.

10. Wells, H. G., and Hedenburg, O. F.: Toxicity of Carotin, J. Biol. Chem. **27**:213, 1916.

11. Cathcart, E. P.: On the Antitryptic Action of Normal Serum, J. Physiol. **31**:497, 1904.

That the albumose fraction of the pneumonic exudate is much more highly toxic than the albumose of normal lung exudate is seen also from the following experiment.

Two rabbits weighing 2,300 and 2,350 gm. were injected intrathoracically after the method of Auld[12] with doses of 0.02 gm. of normal and pneumonic albumose (in 1 per cent. solution, neutralized) per kilogram, respectively. Four hours later the rabbit receiving the pneumonic albumose showed a rise of temperature from 103.4 F. to 105.8 F., marked dyspnea and appeared toxic and morose. The other rabbit showed no effects. The "pneumonic" rabbit was killed and

TABLE 6.—Comparative Toxicity of Albumins and Albumoses from Normal and Pneumonic Human Lung Exudates*

	Normal Exudate			Pneumonic Exudate		
	Weight of Rat, Gm.	Size of Dose, Gm. per Kg.	Result	Weight of Rat, Gm.	Size of Dose, Gm. per Kg.	Result
1. Albumins	132	1.0	Very mild toxic symptoms	128	1.0	Severe dyspnea; recovered, 2 hr.
	145	0.8	Very mild toxic symptoms	137	0.8	Very severe dyspnea; recovered, 1 hour
2. Albumoses	58	1.0	Mild dyspnea	58	1.0	Very severe convulsions; moribund; dyspnea; recovered
	65	0.8	Moderately severe respiratory disturbances	73	0.8	Collapsed
	77	0.6	No symptoms	82	0.6	Severe convulsions with death in 3 hr.†

* Rats were injected intraperitonealy with 1 per cent. solutions of neutralized substances in physiologic sodium chlorid solution.
† Histologic section of the lungs of this rat showed acute hyperemia.

necropsy held on the third day, and sections were made of the lungs and kidneys. The former show that the bronchi are unaffected. There is no exudate and no involvement of the bronchial walls. There is, however, a hyperemia with slight hemorrhagic exudation into the alveoli in scattered areas. There is no consolidation. The predominating change is the hemorrhagic extravasation into the alveoli in certain areas. Histologic sections of the kidneys of this habbit show acute hyperemia, particularly of the glomerulus, accompanied by a slight degree of intratubular hemorrhage. There is also a cloudy swelling of the tubular cells. The picture is that of an early stage of a diffuse nephritis. There are also areas of tubular distention due to the plugging up of tubules.

12. Auld, A. G.: Selected Researches in Pathology, 1901, Ch. 2 and 3. J. and A. Churchill, London.

It was of interest, in connection with our theory as to the rôle of the albumoses in the mechanism of the crisis in pneumonia (discussed later) to determine whether or not a tolerance or immunity to pneumonic albumose could be produced experimentally in animals. For this purpose each of the rats of Table 6 that had recovered after the injection of pneumonic albumose was reinjected intraperitoneally with a dose as large as the first. The rats showed only very mild reactions and none of the toxic symptoms that followed the first dose.

Additional studies were conducted on both normal and pneumonic lungs. Only brief reference can be made to these at this time.

Dried pneumonic lungs on extraction with ether in a Soxhlet apparatus yielded large amounts of lipoidal, nontoxic material. These lipoids when added to a suspension of guinea-pig erythrocytes were deposited on the surfaces of the latter, producing what appeared macroscopically as a cream-colored emulsion. Microscopically, the cells appeared to be undergoing poikilocytosis, but no hemolysis was evident after prolonged incubation. This phenomenon was also obtained with the use of pure lipoid antigen used in the Wassermann reaction for syphilis. On fractionating the lung lipoids into an acetone-soluble and acetone-insoluble fraction, the former was found to be hemolytic, the latter not. Neither fraction, however, produced the poikilocytic phenomenon described. In view of the observation that removal of this lipoidal substance from pneumonic lung renders it more hemolytic, we conclude that its production in the exudate is in response to the injurious action of the hemolytic pneumotoxin. This is in accord with the findings of Cole,[8] that lipoids such as cholesterin and lecithin inhibit hemolysis of erythrocytes by pneumotoxin, and the observations of Bogomolez[13] that excess amounts of lipoids are formed in response to infection by toxin-producing organisms. The lipemia, acidosis and defective glycolysis observed in pneumonia, as well as the decrease in amount of lipase in pneumonic lungs, mentioned by Wells,[14] can thus be traced to the influence of pneumotoxin.

Of the various protein fractions isolated and referred to in the foregoing, none were found to be hemolytic, except the pneumotoxin. The whole pneumonic exudate, as has already been stated, was always hemolytic to a far greater degree than normal exudate — even after one year's storage in a dried state. Solution of the protein fractions in bile, using the technic described for the preparation of pneumotoxin,[9] did not yield hemolytic substances. The hemolytic activity of pneumonic exudates is therefore due to the presence of pneumotoxin as

13. Bogomolez: Ztschr. f. Immunitätsforsch. **8**:35, 1910.
14. Wells, H. G.: Chemical Pathology, Ed. 3, W. B. Saunders Company, Philadelphia, 1918, pp. 413, 560, 78.

well as of various fatty acids, lactic acid and other products of autolysis observed by Lamar.[15]

Using the various protein substances referred to in this paper (excepting pneumotoxin) as antigen, no precipitin reaction was obtained on addition of various serologic types of antipneumococcus horse serum.

No pentose such as observed by Burnett[16] in tissue of rat carcinoma was detected in our preparations.[17] We concluded from all these experiments that other than pneumotoxin (the endocellular toxic protein liberated on dissolution of pneumococci) and the toxic albumose which is the result of the partial digestion of serum albumin during autolysis, no specific toxic proteins are formed during the course of an attack of lobar pneumonia in man.

DISCUSSION

The occurrence of toxic albumoses in pneumonic lungs, in the urine of patients suffering with lobar pneumonia, as well as in the tissues of rabbits having experimental pneumonic septicemia, was demonstrated by Simon,[18] Matthew,[19] Auld[12] and others many years ago. We pointed out their bearing on the mechanism of the crisis in pneumonia in earlier communications (Footnotes 9 and 20). While largely a matter of conjecture, this theory is of sufficient interest to warrant elaboration in the light of the data presented in this paper.

We regard the formation of the pneumonic exudate *in part* due to an increased permeability of the endothelial cells of the lungs for various *normal* serum albumins, globulins, fibrinogen and enzymes, resulting from the injurious action exerted by the pneumotoxin on their cement substance. The toxin is also assumed to act as a lymphagogue.

Other factors which, according to the recent studies of Jacques Loeb, M. Fisher and others,[21] are active in the production of inflammatory edemas, play their usual rôle. Prominent are the impediment

15. Lamar, R. V.: Chemo-Immunological Studies in Localized Infections, J. Exper. M. **13**:1, 1911.

16. Burnett, T. C.: Note on a Toxic Nucleoprotein Obtained from Rat Carcinoma, Proc. Soc. Exper. Biol. and M. **14**:63, 1916.

17. The nucleoprotein fraction of pneumonic lung exudate, which was isolated but not fully studied because of the scarcity of guinea-pigs, also reacted negatively to the orcein test.

18. Simon, O.: Untersuchungen über die Lösungsvorgange der croupösen Pneumonie, Deutsch. Arch. f. klin. Med. **70**: Parts 5 and 6, 1901.

19. Matthes, M.: Arch. f. exper. Path. u. Pharmakol. **36**:437, 1895.

20. Weiss, C.: The Properties of Pneumotoxin and Its Probable Rôle in the Pathology of Lobar Pneumonia, J. M. Res. **39**:103, 1918.

21. Epstein, A. A.: Concerning the Causation of Edema in Chronic Parenchymatous Nephritis, Am. J. M. Sc. **154**:638, 1917.

of lymph outflow caused by the plugging up of the lymphatic channels by clots and leukocytes, increased osmotic pressure and increased hydrophilic properties of the cells due to the action of lactic, formic and fatty acids, enzymes and other products of bacterial (pneumococcic) and cellular metabolism.

The exudate, therefore, is formed from *normal* constituents of the circulating body fluids which have been transferred to and have accumulated in the alveolar spaces. This migration has at least two distinct effects. First, it depletes the blood of its content of serum proteins, which normally has a tendency to be constant, thus producing a diminution in osmotic pressure which is compensated for by a retention of chlorids.[22] Second, it brings to the lung a large excess of material which is foreign to it and which, therefore, would be subject to an *accelerated catabolism* (autolysis) in accordance with the law of mass action, in order to keep the blood proteins constant, to rid the lung of foreign proteins and to restore the physicochemical equilibrium of the body fluids.

We now assume that while the pneumococci proliferating in the body fluids may activate the enzymes that accomplish the autolysis of the fibrin, leukocytes, etc., in the lung,[23] their *toxins* tend to inhibit this autolysis with the production of only partly digested products: albumoses and peptones, such as demonstrated in the foregoing. These toxic products are pyrogenic, as pointed out by Jobling.[24] They act as lymphagogues, aid in further inhibition of autolysis and increase the coagulation time of the blood, as shown by Dochez.[25] Thus we account for what Riesman[26] has called the "metabolic toxemia" of pneumonia.[27] Moreover, the serum of the inflammatory exudate which is known to contain albumoses and peptones was found by Opie[28] to retard the action of the autolytic enzymes of the leukocytes. Weiss, Kolmer and Steinfield[1] also demonstrated the presence in pneumonic exudates

22. Peabody, F. W.: Studies of the Inorganic Metabolism in Pneumonia, J. Exper. M. **17**:71, 1913.

23. A. E. Taylor (Digestion and Metabolism, Lea and Febiger, Philadelphia, 1912, p. 138) points out that bacterial extracts and tissue juices activate proferments, such as pepsinogen.

24. Jobling, J. W., Petersen, W., and Eggstein, A. A.: Serum Ferments and Antiferments During Pneumonia, J. Exper. M. **22**:568, 1915.

25. Dochez, A. R.: Coagulation Time of the Blood in Lobar Pneumonia, J. Exper. M. **16**:693, 1912.

26. Riesman, D.: The Cellular Factor in Infectious Diseases, Tr. College Phys., Philadelphia **36**:271, 1914.

27. We have discussed the cause of the initial toxemia in another communication (Footnote 20).

28. Opie, E. L.: Enzymes and Anti-Enzymes in Inflammatory Exudates, J. Exper. M. **7**:316, 1905.

of substances that hinder the various defensive immunologic reactions of the body fluids.

Simultaneous with the production of the toxic substances thus far alluded to, we have the liberation of various specific antibodies[29] and of bactericidal and phagocytic substances.[30] The demonstration of these in vitro, before the crisis, is difficult and often impossible owing to the presence of excess amounts of antigen (pneumotoxin and albumose), as suggested by the work of Weil.[31] With the production of excess amounts of immune bodies and *particularly of a tolerance or immunity to the toxic albumoses,* the deleterious and autolysis-inhibiting influences are removed.

It is also probable that the continuous action of the toxins, aside from antibody production, changes their primary inhibitory effect on autolysis into an accelerating one. This is suggested by the work of Hess and Saxl.[32] This accelerated autolysis in turn hastens antibody production, as pointed out by Blum.[33] The products of autolysis are now completely digested proteins in the amino-acid stage which are nontoxic and easily absorbed and eliminated.

The equilibrium of the system described is governed by the laws of mass action as suggested by Taylor[23] and more recently by the work of Bayne-Jones.[34] Hence the change from febrile toxemia to the afebrile atoxic state is necessarily an abrupt one — crisis. The period is accompanied by the sudden drop in temperature, relief to toxemic symptoms, a decrease in amount of proteoses in the serum, return to normal coagulation time, and normal protein content of the blood.

The autolysis is probably accomplished by the proteolytic enzymes of the leukocytes. Hartman[35] suggests that the fibrin requires specific antibodies for its removal, but we are inclined to agree with Simon[18] who concludes after thorough chemical investigations that the leuko-

29. Clough, P. W.: The Development of Antibodies in the Serum of Patients Recovering from Acute Lobar Pneumonia, Bull. Johns Hopkins Hosp. **24**:205, 1913.

30. Winternitz, M. C., and Kline, B. S.: Studies on Experimental Pneumonia in Rabbits, IX, J. Exper. M. **21**:320, 1915.

31. Weil, R., and Torrey, J. C.: Immunological Studies in Pneumonia, J. Exper. M. **23**:1, 1916.

32. Hess, L., and Saxl, P.: Einfluss der Toxine auf den Eiweissabbau der Zelle, Wien. klin. Wchnschr. **21**:248, 1908. Ibid., Experimente an autolysierende Organe, p. 486.

33. Blum, L.: Ueber die Antitoxinbildung bei Autolyse, Beitr. z. chem. Physiol. u. Path. **5**:142, 1904.

34. Bayne-Jones, S.: Equilibria in Precipitin Reactions, J. Exper. M. **25**:836, 1917.

35. Hartman, C. C.: The Antigenic Properties of Fibrin (Exudate) to Serum, J. Infect. Dis. **13**:69, 1913. The Antigenic Properties of the Constituents of Pneumonic Exudate, Ibid. **13**:499, 1913.

cytic enzymes will digest the fibrin as well as the other constituents of the exudate. Ascoli[36] is of the opinion that isolysins are formed to remove the cellular constituents. He demonstrated such isolysins after the crisis.

That the pneumotoxin exerts no influence on the lung tissue itself and that the latter plays no part in the formation of the exudate is well known, as mentioned by Wells.[14] Our own observations on the identity of the albumins of normal and pneumonic (exudate-free) lungs substantiates this.

CONCLUSIONS

Biochemical studies of pneumonic exudates obtained from human lungs in the stage of gray hepatization were conducted by the method of anaphylactic sensitization and intoxication of guinea-pigs with various proteins derived from normal and pneumonic lungs, exudates, serums, etc. The following observations were made:

1. Pneumonic exudates contain *at least two* toxic proteins: (1) a specific sensitizing protein apparently identical with the pneumotoxin which is liberated on the dissolution of virulent pneumococci, and (2) an extremely toxic, pyrogenic albumose.

2. There are also present normal serum proteins: serum albumin and serum globulin, leukocytes and fibrin.

3. Neither undigested pneumococcic protein nor albumin derived from the lung tissue, possessing sensitizing powers, are demonstrable.

4. The globulin fraction of human pneumonic exudate is nontoxic and identical with similar normal globulin.

5. The albumin fraction of pneumonic exudate is toxic and possesses marked specificity. This is ascribed to the digestive action of the enzymes of the exudate.

6. The albumose fraction is far more toxic. Dyspnea, convulsions and death follow the intraperitoneal injection into white rats of doses of 1 gm. per kilogram. Intrathoracic injection into rabbits of doses of 0.02 gm. per kilogram produces a rise in temperature, dyspnea, a hemorrhagic extravasation into the alveoli; of the lung and an acute diffuse nephritis. On repeated intraperitoneal injections of this albumose into rats, a tolerance to it can be established.

7. Large amounts of ether-soluble, nontoxic, hemolysis-inhibiting substances were extracted from pneumonic lungs. These are assumed to have the power of neutralizing the hemolytic activity of the pneumotoxin in vivo.

8. The formation of the exudate in pneumonia is considered to be *in part* due to an increased permeability of the endothelial cells of

36. Ascoli, M.: Isoagglutinine und Isolysine in menschlicher Blutsera, München. med. Wchnschr. **48**:1239, 1901.

the lung for various *normal* serum albumins, globulins, fibrinogen and enzymes as the result of the injury exerted by the pneumotoxin on their cement substance. The toxin is also regarded as a lymphagogue. It hinders the autolysis of the exudate and the favorable action of antipneumococcus immune bodies and thus produces toxic autolysis-hibiting, pyrogenic albumoses. With the development of excess amounts of specific antibodies, of bactericidal and phagocytic substances, and of a tolerance to the toxic albumoses, the deleterious influences of the toxins and albumoses are neutralized. Autolysis of the exudate is now unhindered and the products are nontoxic amino-acids. The equilibrium of this system being governed by the laws of mass action, the change from febrile toxemia to the afebrile, atoxic state is necessarily an abrupt one — *crisis*.

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CORRECTION

Attention is called to errors in the legends of the charts illustrating the article in the February, 1919, number (p. 235) by A. O. Gettler and R. Oppenheimer on "Differentiation of Nephropathies, Cardiopathies and Allied Conditions by the Determination of Physical Constants." The legend of Chart 1 should read "Ash" instead of "Refractive index;" Chart 2 should be "Freezing point" instead of "Solids"; Chart 4 should be "Solids" instead of "Freezing Point"; Chart 5 should be "Refractive index" instead of "Ash." We regret that these errors occurred. Corrections have been made in the authors' reprints.